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LABORATORY METHODS FOR THE DETECTION OF MILK FROM COWS INFECTED WITH MASTITIS

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The common occurrence of udder infections in dairy cows has motivated investigators to develop reliable laboratory methods for detecting milk which has been produced wholly or in part by animals suffering from such an infection. It is not the purpose of this paper to discuss the public health aspects of consuming milk from diseased udders, but merely to present data and discussion which may assist in determining the reliability of available methods for detecting milk produced by cows suffering from this condition. The data presented in this paper were secured from a large herd under efficient management. The cases of mastitis reported are usually of the subclinical or chronic type which are so prevalent and may exist for years without the dairyman's knowledge. However, occasional cases of acute mastitis occurred and these were also included.

HISTORICAL

The prevalence of chronic streptococcic mastitis in cows can well be judged by the report of Rosell (24), who has found that it affects from 20 to 50 per cent of high milk producers in different countries, and causes a greater loss to the milk industry than any other cattle disease. He found that chronic streptococcic mastitis actually constitutes at least 98 per cent of all cases in high-producing cows.

Methods employed for the detection of milk from infected udders have generally been developed on the basis of (1) direct microscopic examination of milk to determine the presence of abnormal cellular constituents—streptococci, leucocytes, fixed tissue cells, etc.; (2) cultural tests to reveal the numbers and kinds of bacteria present; and (3) chemical tests which would reveal the presence of abnormal constituents in the milk. It is not the intention of this paper to present a complete bibliography on the various phases of this subject, but merely to cite a few pertinent references which will indicate some of the previous results and methods of attack.

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The direct microscopic bacterial examination of milk for detecting udder infection is necessarily limited to freshly drawn samples from individual cows. In cases where the infection is not extremely severe it would probably be necessary to use samples from the individual quarters. Breed and Brew (5) recommended the microscopic examination of milk as a means of detecting high counts due to udders infected with streptococci.

The occurrence of an abnormal number of leucocytes and fixed tissue cells in milk has also been considered as an indication of infection. Opinion differs on this subject; for example, Breed (3, 4) reported obtaining apparently normal milk strippings from a single teat which contained 54,000,000 leucocytes per cubic centimeter; no streptococci or other undesirable bacteria could be found in this milk. In contrast to these findings Cherrington *et al.* (7), using the Prescott and Breed (23) method, concluded that "Milk from normal udders usually contains less than 50,000 leucocytes per cubic centimeter, whereas milk from infected udders almost invariably contains more than 100,000 leucocytes per cubic centimeter."

More recently Hucker *et al.* (12) studied the incidence of sub-clinical mastitis in dairy districts as well as methods of detecting this condition in 221 cows condemned by the tuberculin test. In their excellent report they conclude that "More than 3,000,000 cells per cubic centimeter almost invariably indicate a present or past infection with streptococci." In a more recent article Hucker (13) says "Ninety per cent of the normal quarters, *i.e.*, quarters free from scar tissue, showed less than 60,000 cells per cubic centimeter, while a great majority, *viz.*, 87 per cent, never gave a higher count than 30,000 cells per cubic centimeter."

Cultural tests generally have been used to determine the sanitary quality of market milk samples. Samples of milk used to determine the presence of udder infections should be taken aseptically from individual cows and cultured immediately. Hastings and Hoffman (9) studied two cows which produced milk containing large numbers of bacteria and leucocytes. They believed the animals had suffered from mastitis at some previous time and had become chronic carriers of bacteria. This probably explains the origin of the so-called "high count" cows. Cherrington *et al.* (6) present data showing that "bacterial counts pertaining to healthy cows show that 86 per cent of the counts on plain agar and 78 per cent on blood agar were less than 1,000 per cubic centimeter, whereas 69 per cent of the samples from infected udders contained more than 2,000 bacteria per cubic centimeter on either plain or blood agar." They conclude that "Bacterial counts on blood agar were more reliable for detecting udder infections than those on plain agar because plain agar often failed to produce distinct colonies from the organisms which were responsible for the infection."

Hucker *et al.* (12) used not only the cell count as mentioned above but also "number of colonies appearing on veal infusion agar with and with-

out blood, number of colonies appearing on Burri slants, a direct microscopic examination, microscopic examination subsequent to incubation over night at 37° C., the amount of chlorides, lactose and catalase present and the reaction (brom-thymol-blue)."

Various modifications of the fermentation and curd tests have been used on market milk but they apparently never have been studied with the view of adapting them to the detection of udder infections. Orla-Jensen (22) suggests the rennet test to determine if milk has been drawn from diseased udders. More recent work by Hill (11) on the curd characteristic of milk shows that extreme variation exists between the curd characteristics of milk from individual cows within a given herd.

Chemical tests which would reveal the presence of substances present in milk from infected udders yet lacking in normal milk have been sought extensively. These tests generally have failed because of the complex composition of normal milk. Rosell (24) reports that the most important chemical changes in milk as the result of infection are (a) an increased pH, (b) an increase in catalase due to an increase in leucocytes, (c) increase in chlorine, (d) increase in heat coagulable albumen, and (e) decrease in potential acidity, lactose, casein, etc. He says "their determination is just as important as a bacteriological examination."

Baker and Van Slyke (2), Baker and Breed (1), Tweed (25), Hucker *et al.* (12), and Udall and Johnson (26) found a close relationship between decreasing hydrogen-ion concentration of the fresh milk and an abnormal number of leucocytes. They attribute the decreasing hydrogen-ion concentration to the entrance of the alkaline substances of the blood into the milk. The amount of chlorides in milk has been considered significant by Hammer & Bailey (8), Hayden (10) and Hucker (12). Hucker (14) summarizes: "Under general conditions it is assumed that all milk showing more than 0.14 per cent chlorides has been derived from infected udders." Hayden (10) developed several rapid tests for detecting milk which contained more than 0.14 per cent chlorine.

Enzymes also have been studied—oxidases and reductases and the very common catalase. The common occurrence of oxidases in normal milk precludes its application. However, the action of reductase has been employed in the well-known methylene blue test. This method has been used extensively for the grading of market milk, but its value for detecting milk from infected udders never has been adequately determined. Catalase, which occurs in abundance in blood and the tissues of the body, is present only to a slight degree in normal milk. Since infections liberate constituents of the blood into the milk in such quantities that the hydrogen-ion concentration is appreciably altered, it should only be expected that such milk serum mixtures would have an abnormal catalase content. Orla-Jensen (22) states, "Milk drawn from cows with diseased udders or from cows

which are approaching the end of the lactation period or colostrum, will liberate large amounts of oxygen from hydrogen peroxide." He employed a catalase test which he interpreted as a catalase number. He said fresh milk from healthy cows would not yield more than 2.5 cubic centimeters of oxygen from a mixture of 15 cubic centimeters of milk and sufficient hydrogen peroxide (1 to 3 per cent) to fill a 20 cc. test-tube. The number of cubic centimeters of gas produced at 20 degrees to 25 degrees Centigrade in six hours was taken as the catalase number. Hucker (12) used the catalase test and after stating that the test was very sensitive, concluded "that it is neither more delicate nor more accurate than other tests which are more easily made." Extensive studies on the catalase test have been made by Morgulus, Beber, and Rabkin (18, 19, 20), who point out the lack of knowledge of the theory of catalase activity.

A simple, quick, qualitative or quantitative colorimetric method for detecting pus in milk would be of great service for routine examination. Clinical methods have been developed for the detection of pus in urine, etc. These tests are usually based on the microscopic demonstration of large numbers of leucocytes. However, in cases where it is impossible to make a microscopic examination, chemical tests have been applied. Vitali's test and Donne's test have been used extensively for the detection of pus in urine. It would appear that the successful application of these tests to the detection of pus in urine would justify efforts to apply these methods to the detection of pus in milk. Their application to milk, however, is rendered difficult because of its complex composition. Kastle (15), in reviewing the chemical tests for blood, cites references which indicate that it is the iron of the hemoglobin and its iron-containing derivatives that are responsible for the guaiacum (Vitali's) reaction. He lists pus as a substance that does not react with guaiacum or other chromogenic substances employed in hematologic investigations, either alone or with hydrogen peroxide. Other chromogenic substances listed by Kastle that have been employed for this test are guaiacomic acid, aloin, benzidin, the leuco-base of malachite green, and phenolphthalein.

The constituents of pus serum may serve also as an index of pyogenic infections. These might include degradation products of bacteria, fixed tissue cells, leucocytes, or serum. Mathews (17) says, "There are in nearly all cells, and in possibly all cells, autolytic enzymes (endocellular enzymes) or nucleases, which decompose nucleic acids into its various constituents." Wells (29) describes the autolytic action taking place in necrosis, decomposing nucleoproteins of the nuclei and liberating nucleic acids. Later the nucleic acids are further decomposed into purine bases. Von Fellenberg (27) in reporting on the determination of purine bases in foods showed that animal products such as fish, poultry, beef, etc., as well as organs and glands were richest, while blood was poorer and milk and eggs

practically free. Mandel and Levine (16) show that glucothionic acid derived from leucocytes is also present in pus. Wells (28) states that pus also contains other constituents of the leucocytes, particularly lecithin, cholesterol, fats (and soaps), cerebrin, "jecorin," and glycogen, and also the usual components of the blood serum as well as some small quantities of pigment derived from decomposed red corpuscles. The authors have found no references where tests based on these principles have been employed for the detection of pus in milk. Experiments are now under way with the hope of applying these principles.

PURPOSE OF THIS INVESTIGATION

The purpose of this investigation is to determine the merits of available methods for the detection of milk from infected udders. This study was undertaken with the hope of finding methods which would have sufficient merit to justify their adoption by dairy inspectors and laboratory technicians for routine testing of milk. Emphasis has been placed on the chemical composition of milk because of the desirability of having a quick chemical method that may be used by dairymen and inspectors with limited laboratory facilities. The methods should have sufficient reliability to detect the less severe cases of udder infection.

The cows used in this experiment belong to the dairy herd of the University of Idaho. They are free from tuberculosis and Bang's abortion disease as attested by repeated tests.

METHODS

Only middle milk was used in the following tests because of the wide variations in the numbers of bacteria generally reported in fore milk and strippings.

The numbers of leucocytes in milk were determined by the direct count method developed by Prescott and Breed (23). This method has been emphasized in a previous publication (7).

Enzymatic products were studied by making such tests as would demonstrate the presence of catalase and the oxidizing enzymes. The catalase test was run after the method described by Orla-Jensen (22). The guaiac test and the benzidin test for oxidizing enzymes were made according to standard clinical methods. The phenolphthalein test, which is also an oxidase test, was made according to the method of Kastle (15).

The presence of serum was demonstrated by determining the hydrogen-ion concentration of the milk with the quinhydrone electrode and the Brom cresol purple method of Baker and Van Slyke (2). Chlorides were determined according to field method No. 3 of Hayden (10), which merely indicates whether the chlorine content is above or below 0.14 per cent.

The numbers of bacteria were determined by the standard plate method,

using plain nutrient agar and also blood agar containing 5 per cent defibrinated horse blood and 1 per cent dextrose.

EXPERIMENTAL

Table 1 presents data portraying typical conditions found in milk from normal udders and from udders showing sub-clinical and acute mastitis. This table is presented to show the relative value of each method and to show the great contrast that exists in the quality of milk from the various quarters of the same udder. The data presented here depict definite clinical results which would characterize a normal udder or a diseased udder. Even the acute or clinical mastitis presents a different picture from the sub-acute or chronic mastitis. This however is not exactly a true picture of the problem for actually there appears to be varying degrees of intensity from the most minute indication to the severe acute or clinical type in which the udder becomes inflamed, the milk flow stops almost entirely—a spongy, amber-colored, purulent mass is discharged instead—and the animal is in great discomfort. Then, too, no single determination can give a true picture of the case, for we find that some cows having sub-acute cases frequently develop the acute stage only to subside to the sub-acute again, whereas several animals which have been under observation for five years have never had an acute attack during this time and yet have maintained constantly a typical picture of sub-clinical mastitis. During the course of these studies several animals have developed acute mastitis which has disappeared entirely, but generally an udder which is once badly infected will continue to produce milk of high leucocyte and catalase content after the swelling has disappeared and the milk has returned to normal appearance.

Udall and Johnson (26) claim that a physical examination of the udder rates above any other single method. They point out that pathological changes in the udder are located more uniformly and certainly by this method because of the tissue change fibrosis—scar tissue which develops as the result of infection. They point out that bacteriological methods may fail because grossly diseased udders do not give off streptococci constantly. During the course of these studies the cows have been under constant veterinary supervision, and detailed physical examinations of the udders have been made. These examinations have demonstrated the presence of fibrosa and lack of uniformity in consistency of the quarters in more than half of the animals under observation; however, some of these animals are high-producing cows whose milk is entirely normal, according to the chemical and bacteriological methods used in the laboratory. These studies indicate that the physical examination of the udder possesses merit for locating tissue change due to disease, which probably would serve as a practical basis for segregating diseased cows. Chemical and bacteriological methods alone, however, can establish the actual quality of the milk produced; practical

TABLE 1

Table Showing Simultaneous Tests On Milk From Individual Quarters

COW NO.	QUARTER NO.	BACTERIA PER CC.		TYPE OF INFECTION	LEUCOCYTES PER CC.	CATALASE TEST CC. OXYGEN	PH	CHLORIDE TEST	CURD TENSION (GRAMS)
		Plain agar	Blood agar						
				Normal Udders					
	1	150	100		11,000	.75	6.6–	0	39
	2	150	300		11,000	1.00	6.6–	0	46
89	3	50	200		22,000	1.50	6.6–	0	46
	4	200	500		22,000	2.00	6.6–	0	40
	1	0	100		88,000	2.00	6.6+	++	22
	2	0	0		88,000	2.00	6.6+	+	27
*4X	3	0	300		22,000	1.25	6.6–	0	22
	4	800	900		44,000	3.00	6.6+	+	22
	1	50	0		22,000	1.50	6.5	0	102
	2	50	200		44,000	1.00	6.5	0	87
170	3	100	100		88,000	1.00	6.5	0	113
	4	550	0		22,000	1.50	6.5	0	93
	1	300	300		22,000	0.75	6.5	0	65
	2	200	300		22,000	0.75	6.5	0	72
172	3	0	200		22,000	0.50	6.5	0	80
	4	200	0		22,000	0.75	6.5	0	67
	1	0	0		22,000	0.50	6.5	0	55
	2	0	0		44,000	0.25	6.5	0	50
165	3	0	0		22,000	0.25	6.5	0	72
	4	100	100		44,000	0.75	6.5	0	55
	1	1,000	0		22,000	0.75	6.5	0	75
	2	700	0		44,000	1.50	6.5	0	80
175	3	100	0		22,000	0.75	6.5	0	85
	4	100	200		22,000	2.00	6.55	0	75
				Acute Mastitis					
	1	0	0		176,000	8.50	6.7+	++++	20
	2	900	5,100	Diplococcus	10,000,000	18.00	7.3	++	0
1X	3	200	100		22,000	2.50	6.9	+	18
	4	0	0		44,000	5.25	6.9	+	18
	1	200	100		224,000	8.25	6.7	+	43
	2	150	0		176,000	6.00	6.7	0	57
164	3	100	100		176,000	8.50	6.7	0	55
	4	2,000	1,800	Diplo. & Strep.	2,508,000	17.00	7.0	++++	0
	1	3,200	250,000	Streptococcus	858,000	6.25	6.7+	0	67
	2	100	600	Streptococcus	858,000	5.00	6.7–	0	67
151	3	41,000	60,800	Staphylococcus	7,480,000	14.50	6.7–	0	63
	4	700	2,100		17,600,000	.....	7.0+	++++	0

*It is very probable that this cow had an extremely mild infection.

TABLE 1.—(*Continued*)
Table Showing Simultaneous Tests On Milk From Individual Quarters

COW NO.	QUARTER NO.	BACTERIA PER CC.		TYPE OF INFECTION	LEUCOCYTES PER CC.	CATALASE TEST CC. OXYGEN	pH	CHLORIDE TEST	CURD TENSION (GRAMS)
		Plain agar	Blood agar						
				Acute Mastitis					
	1	600	2,600		2,068,000	11.00	7.0	++++	18
	2	150	1,000		2,024,000	9.75	7.0	++	18
61	3	0	100		1,980,000	12.50	6.8	0	27
	4	0	0		572,000	7.00	6.8	0	23
	1	0	1,100		1,628,000	7.50	6.9+	++++	22
	2	150	0		572,000	5.50	6.6+	+	28
98	3	100	200		924,000	4.75	6.7	+	33
	4	0	10,500	Streptococcus	1,012,000	18.00	6.9	++	12
				Sub-clinical Mastitis					
	1	0	0		2,200,000	10.00	6.7	0	46
	2	100	0		176,000	1.00	6.7	0	75
150	3	4,500	32,000	Staphylococcus	9,680,000	17.50	6.9	0	30
	4	0	0		22,000	.50	6.7	0	85
	1	0	450,000	Streptococcus	24,200,000	.....	6.2	++++	12
	2	100	700		880,000	14.25	6.9	0	16
78	3	200	800		5,280,000	18.00	6.8	0	12
	4	100	900		2,420,000	14.00	6.9	0	9
	1	200	1,100		308,000	16.25	6.7	0	41
	2	100	0		616,000	8.25	6.7+	0	34
52	3	0	100		132,000	18.00	6.9	+	32
	4	0	1,500	Staphyococcus	10,000,000	18.00	7.0	0	12
	1	50	100		22,000	2.0	6.8	0	49
	2	0	0		22,000	1.25	6.6	0	63
160	3	300	700		22,000	1.0	6.5	0	72
	4	50	0		858,000	11.0	6.6	0	37
	1	100	100	Streptococcus	880,000	3.75	6.5	+	47
	2	1,000	1,000		1,056,000	9.5	6.5	+++	27
24X	3	300	100		704,000	4.0	6.5	+	50
	4	0	11,600	Streptococcus	396,000	6.0	6.5	++++	27
	1	0	3,400	Streptococcus	88,000	8.0	6.8	0	18
	2	1,200	4,200	Streptococcus	1,000,000	12.25	6.8	0	15
88	3	1,300	5,400	Streptococcus	572,000	7.00	6.7+	0	25
	4	350	800		1,760,000	14.50	6.9	0	15
	1	200	5,900	Streptococcus	2,200,000	9.00	6.9–	0	15
	2	100	5,100	Streptococcus	660,000	9.00	6.8+	0	15
167	3	100	400		748,000	12.50	6.8+	0	14
	4	300	700		1,012,000	12.00	6.9+	0	15
	1	0	0		44,000	0.50	6.6	0	22
	2	250	400		22,000	1.50	6.6–	0	23
18X	3	400	100		22,000	0.25	6.6–	0	29
	4	200	200		880,000	9.50	6.6+	0	23

methods and standards can be developed which would detect market milk from diseased udders. Thus they serve the double purpose of insuring the public a milk supply free from this objectionable condition and furnishing an incentive for dairyman to use such sanitary methods as are necessary for the control of the disease.

LABORATORY METHODS

The efficiency of the several tests used must be established by determining the extent to which the results are in agreement. Most of the cases reported here have been under observation from three to five years, and the data presented can be regarded as typical of the voluminous observations and determinations that have been made.

DIRECT MICROSCOPIC EXAMINATIONS

The authors agree with Hucker *et al.* (12) that direct microscopic examination of freshly drawn milk for streptococci has limited significance in indicating infected udders of the sub-clinical type, for only rarely does milk as drawn from the udder carry sufficiently large numbers of streptococci to be detected microscopically without at the same time being abnormal in appearance. Hucker *et al.* (12) were able to detect streptococci in milk which harbored few organisms by incubating the samples at 37 degrees Centigrade over night before making the final examination. The authors have employed this method with carefully drawn samples and found it effective for bringing about an enrichment of the organisms, especially upon the addition of 1 per cent dextrose. The mere presence, however, of long chain streptococci in incubated samples cannot be taken as positive evidence of udder infection. It is of value only when correlated with other findings.

PLAIN NUTRIENT AGAR AND BLOOD AGAR COUNTS

The counts on plain agar and blood agar do not always agree, but in general it may be said that the blood agar counts are much higher than those on plain agar. Plain agar is poorly suited for the detection of streptococcic mastitis because the colonies formed are usually so small that they are not detected with the naked eye. During the course of this work it was found advantageous to add 1 per cent dextrose to the blood agar because it caused the colonies to grow large enough to be counted readily. The principal objection to the dextrose is that it interferes with the hemolytic characteristics of the organisms. It will be observed that the agar count by either of these methods is not a satisfactory index of udder infection because occasionally no colonies are formed when it is very apparent from other tests that mastitis does exist. This probably is because the diseased condition was not active at that time. Therefore, it would be impossible to

say that any given number of bacteria are permissible and normal, and that more than this number means that the quarter from which the milk is drawn is diseased.

SIGNIFICANCE OF THE NUMBER OF CELLS

It is impossible to reconcile the divergent views expressed concerning the significance of the cell content of milk. These data were secured by taking a standard loopful of milk and spreading it over an area of one square centimeter. This film is stained by the rapid (one solution) method of Newman (21) and counted under the oil immersion objective of the microscope. The experience of this laboratory has developed reliance in this method. Though millions of leucocytes are found in the clinical or acute mastitis, they are also quite numerous in sub-clinical cases. The results of this paper are in agreement with the previous publication (7), which pointed out that more than 100,000 leucocytes per cubic centimeter is a good indicator of udder infection.

CATALASE TEST

This test has been run according to the method described by Orla-Jensen, in which a 20 cc. capacity test-tube is filled with 15 cubic centimeters of milk and sufficient 3 per cent peroxide to fill the tube. A rubber stopper containing a piece of bent glass tubing is inserted and the tube is inverted. This simple test has proved very satisfactory. An examination of the data shows a very close agreement to exist between this test and the cell or leucocyte count. Orla-Jensen claimed that milk from healthy cows would not yield more than 2.5 cubic centimeters of oxygen according to his technic. The data presented here conforms well to this standard.

TABLE 2

Relation of Serum and Dilution to Catalase Test and Curd Tension

SAMPLE TESTED	CURD TENSION (GRAMS)	CATALASE TEST (CC. OF OXYGEN)	TIME OF REACTION
Normal milk	111	0.5	6 hrs.
Milk + 5% water	116		
Milk + 5% serum	90	12.0	6 hrs.
Milk + 10% water	100		
Milk + 10% serum	88	16.0	6 hrs.
Milk + 15% water	85		
Milk + 15% serum	65	20.0*	45 min.
Milk + 20% water	80		
Milk + 20% serum	64	20.0*	17 min.
Milk + 50% water	40		
Milk + 50% serum	7	20.0*	4.5 min.
Serum (fresh)		20.0*	1.5 min.

*Complete displacement of liquid.

Table 2 shows the close relationship which exists between the amount of serum which gets into the milk and the catalase test. The serum used in this test was fresh, clear, straw-colored serum, quite free from blood cells. Blood cells contain such a great catalase content that when one drop of whole blood is added to 15 cubic centimeters of water or milk, and peroxide is added, complete displacement of the liquid by oxygen will take place. These data indicate the great catalase content of blood serum. The simplicity and reliability of the catalase test recommend it as a routine test for locating active infection in the cow's udder.

H–ION CONCENTRATION

Both colorimetric and electrometric methods have been employed for the determination of the H-ion concentration. The data reported here were obtained by using the quinhydrone electrode. The H-ion concentration appears to be poorly suited to the detection of sub-clinical mastitis. Occasionally, as in the case of cow No. 78 (Table 1), the milk from a diseased udder actually is more acid than normal. Generally, however, the H-ion concentration is less in milk from disease udders. In sub-clinical mastitis this difference frequently is so small as to be impossible to interpret. In acute mastitis the milk generally is alkaline; however, it is usually so abnormal in appearance under these conditions that there is no necessity for clinical identification. The H-ion concentration has limited merit as a clinical method for the detection of mastitis because the variation in reaction between milk from normal udders and udders showing sub-clinical mastitis is neither consistent nor wide enough in range to permit accurate interpretation.

SIGNIFICANCE OF EXCESSIVE CHLORINE CONTENT

The rapid test No. 3 reported by Hayden (10) was used during this investigation. This test is based on the principle that the presence of more than 0.14 per cent chlorine in the milk indicates that blood serum is entering the milk. This test is simple and easily interpreted. An examination of Table 1 shows that it is generally positive in acute mastitis, and questionable in cases of sub-clinical mastitis. Differences in the normal salt concentration of the milk from individual cows and also the actual amount of salt being consumed by the cow, as well as the degree of the disease, operate to mask the value of this test.

CURD TENSION

The curd tension was measured in grams according to the method of Hill (11). The accuracy of the spring balance used in these tests was limited because it was marked off in 5-gram divisions. However, the duplicate readings usually checked within 5 grams, which was adequate for the purpose of this experiment. It will be observed that normally there is a great

difference in the curd tension of milk from different cows. There is also a small normal variation in the curd tension of milk from the different quarters of the same udder. However, an acute case of mastitis often completely destroys the coagulating power of the milk. Sub-clinical mastitis similarly results in the lowering of the curd tension. Table 2 shows the comparative effect of added blood serum and water upon the curd tension of milk. These data reveal that blood serum has a much greater effect in reducing the curd tension than the same degree of dilution by water. A study of these data suggests the advisability of always running a catalase test on any sample of milk which is being tested for curd tension with the view of using the milk for infant feeding.

TYPE OF ORGANISMS PRESENT

Microscopic examination of thousands of stained preparations made from blood agar plates and films of fresh milk and incubated milk shows that nearly all cases of mastitis encountered during these studies were streptococcic. Occasionally a staphylococcus is found, and in one case one of the authors (6) encountered *Pseudomonas aeruginosa* as the causative agent.

CLINICAL TESTS

Chemical methods regularly employed for the detection of pus in urine have been tried on milk which was known to originate from cases of subclinical mastitis. Vitali's, Donne's, the benzidin and phenolphthalein tests have all been uniformly negative. It is possible that future experiments based on the detection of nucleic acids or their derivatives may be employed successfully to locate pyogenic infections in the cow's udder.

TESTS APPLIED TO MARKET MILK SAMPLES

Table 3 shows the results of applying these tests to retail milk samples picked up by the dairy inspector in his regular tour of duty. The bacterial count here has little significance except to indicate the general sanitary quality of the milk. The significance of leucocytes in the milk from individual cows has been shown previously. The possible application of standards based on such results to the control of market milk can well be ascertained from the results in table 3. If 100,000 leucocytes per cubic centimeter were accepted as a limit, then only 20 of the 54 samples could be retailed. The catalase test shows a close relationship to the leucocyte content except in the case of pasteurized milk, where the catalase is evidently destroyed. The pH value of milk apparently has no significance when applied to retail milk samples. The increased pH values characteristic of milk from infected quarters is lost by dilution with normal milk. It is significant to observe that chlorine in excess of 0.14 per cent was en-

TABLE 3

Tests Made on Retail Milk Samples

DAIRY NO.	BACTERIA PER CC.		LEUCOCYTES PER CC.	CATALASE TEST CC. OXYGEN	pH	CHLORIDE	CURD TENSION (GMS.)
	Plain agar	Blood agar					
1	9,000	10,000	414,000	5.5	6.5	+++	20
2	18,800	39,200	1,380,000	1.0	6.5	–	38
3	23,000	20,800	3,542,000	8.0	6.5	–	50
4	38,000	23,000	1,104,000	4.0	6.5	–	37
5	11,400	9,300	46,000	2.25	6.5	–	100
6	48,200	62,000	92,000	1.75	6.5	–	35
7	9,000	24,000	92,000	3.75	6.5	–	65
8	3,800	5,300	92,000	2.25	6.5	–	67
9*	2,700	300	920,000	0.25	6.5	–	36
10*	12,000	15,800	184,000	0.25	6.5	–	39
11	131,000	43,000	690,000	6.75	6.5	+	101
12	1,000	4,400	23,000	5.0	6.5	–	43
13	7,200	6,500	552,000	6.25	6.5	++	65
14	13,400	5,800	23,000	0.5	6.5	++	48
15	6,000	16,000	230,000	3.5	6.5	+	46
16	21,000	19,800	276,000	3.75	6.5	–	45
17	1,800	3,000	230,000	3.75	6.5	–	37
18	32,000	31,000	138,000	4.00	6.5	–	47
19	12,950	15,000	138,000	3.00	6.5	–	47
20	43,400	35,000	92,000	3.5	6.55	–	50
21	35,000	214,000	484,000	4.0	6.5	–	56
22	27,000	41,600	176,000	2.5	6.5	–	55
23	6,050	7,100	308,000	3.0	6.5	–	50
24	35,500	120,000	2,860,000	8.75	6.5	–	63
25	4,400	3,800	484,000	4.0	6.5	–	44
26	6,450	5,200	176,000	3.25	6.5	–	61
27	4,450	5,400	132,000	3.50	6.5	+	50
28	1,750	1,800	88,000	2.75	6.5	–	82
29	1,500	2,400	88,000	2.50	6.5	–	52
30	2,350	5,900	836,000	4.50	6.5	–	44
31	1,250	1,800	308,000	4.50	6.5	–	40
32	13,500	18,000	132,000	2.0	6.5	–	45
33*	12,000	33,600	88,000	0.25	6.5	–	58
34*	850	20,400	484,000	0.25	6.5	–	40
35*	500	700	44,000	0	6.6	–	49
36	12,600	5,600	132,000	2.00	6.6	–	80
37	5,650	12,800	220,000	2.50	6.5	–	55
38	5,100		934,000	3.5	6.5	–	91.0
39	7,100		46,700	1.5	6.5	–	57.5
40	8,300		934,000	4.0	6.5	–	37.0
41	2,300		186,000	4.5	6.5	+	50.0
42	2,700		46,700	1.25	6.5	–	60.0
43	73,000		46,700	1.5	6.5	–	57.0
44	42,000		93,400	3.75	6.5	–	37.5
45	4,100		93,400	1.5	6.5	–	30.0
46	16,400		93,400	2.0	6.5	–	35.0
47	27,000		93,400	1.5	6.5	–	25.0
48	14,800		1,961,000	4.75	6.5	–	47.5
49*	400		93,400	–	6.5	–	37.5
50	21,400		46,700	2.30	6.5	–	45.0
51	18,400		130,000	4.25	6.5	+	45.0
52	132,000		1,670,000	5.25	6.5	–	18.0
53	3,900		934,000	7.50	6.5	–	45.0
54*	2,700		93,400	–	6.5	–	37.5

*Pasteurized milk.

countered in milk which most certainly came from badly infected herds; however, it must also be observed that many samples showing excessive leucocyte and catalase content did not contain an excess of chlorine. Curd tensions vary over a wide range. Some samples possessing high leucocyte and catalase content possess high curd tensions, whereas other samples may possess low leucocyte and catalase content along with a low curd tension. These data again indicate the desirability of determining the presence of udder infection simultaneously with the curd tension.

SUMMARY

1. Acute mastitis in cows usually is easily recognizable. The affected quarters are intensely inflamed and the cow suffers much discomfort. The milk from these quarters is characterized by producing abundant colonies on dextrose blood agar though the count on plain agar may appear normal. The milk usually contains clots and its consistency is watery—in extreme cases the solids completely separate into a spongy mass which floats in an amber-colored serum. Milk from these quarters contains millions of leucocytes per cubic centimeter; the catalase content is extremely high, usually producing 10 cubic centimeters or more of gas according to the technic here used; the hydrogen-ion concentration decreases until the milk is neutral or slightly alkaline. However, isolated cases are found where the H-ion concentration actually increases; the milk usually contains chlorine in excess of 0.14 per cent, and the curd tension according to Hill's method is almost entirely destroyed.

2. Chronic or sub-clinical mastitis is the most common form. This type of infection is commonly so mild that it passes without recognition. The udder appears superficially normal; flakes or clots may appear occasionally but the milk usually appears normal. This condition is very common in dairy herds. The laboratory detection of this milk is best accomplished by examining the milk from individual quarters of the udder. In this type of mastitis the bacterial count often appears normal on plain agar; dextrose blood agar, however, usually reveals an abnormally high count. The leucocyte count is in excess of 100,000 per cubic centimeter, and the catalase test usually produces 2.5 cubic centimeters or more of gas, according to the technic here used; the H-ion concentration is usually only slightly reduced; the chlorine content of the milk is commonly normal; and the curd tension according to Hill's method is usually reduced.

3. The addition of clear fresh blood serum from blood cells to milk increases the catalase content markedly. The addition of serum also causes a decrease in the curd tension. Blood serum has a much greater effect in reducing the curd tension than the same degree of dilution by water.

4. Chemical tests regularly employed for the detection of pus in urine

have been negative when applied to milk known to originate from cases of sub-clinical mastitis.

5. The physical examination of the udder possesses merit for locating tissue change due to disease; however, chemical and bacteriological methods alone can actually establish the quality of milk which is produced.

6. Retail milk samples when subjected to these tests showed that 33 of the 54 samples tested contained catalase in sufficient quantities to produce 2.5 cubic centimeters or more of gas and 34 of the 54 samples contained more than 100,000 leucocytes per cubic centimeter. The discrepancy between the number of samples showing excessive leucocytes and catalase is due to the pasteurized samples in which the catalase has been destroyed. The H-ion concentration was normal in practically all samples. Only 8 of the 54 samples contained chlorine in excess of 0.14 per cent. The curd tension of herd samples apparently had no relation to the number of leucocytes or to the catalase test.

7. According to this study it appears safe to conclude the leucocytes in excess of 100,000 per cubic centimeter and catalase in sufficient quantity to produce 2.5 cubic centimeters of oxygen or more, according to the method reported by Orla-Jensen, are reliable indices of udder infection.

CONCLUSION

Of all laboratory methods employed in these studies for the detection of sub-clinical mastitis, the leucocyte and catalase content of milk serve as the most reliable indicators.

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RATE OF PASSAGE OF INERT MATERIALS THROUGH THE DIGESTIVE TRACT OF THE BOVINE*

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A knowledge of the rate of passage of inert materials through the digestive tract is of importance in digestibility studies. This information is necessary to determine how long feeds may exert an influence where rations are changed from one feeding régime to another in order that proper interpretation may be given to the results. The development of a method for determining this rate of passage also offers a means for investigating the comparative costive or laxative effect of feeds.

Hoelzel (1) studied the rate of passage of inert materials through the digestive tract of rabbits, guinea-pigs, dogs, cats, albino rats, white mice, pigeons, one monkey, one hen, and one man. The test materials included rubber, cotton thread (knots), seeds, glass beads, pieces of aluminum, steel, silver, and gold. The rate of passage of these inert materials was found to be more or less proportional to the specific gravity of the test materials, the heavier materials passing more slowly than the lighter ones. The rate of passage also varied considerably in the different species and individuals.

Burnett (2) used 50 cc. of French millet seeds in studies with man, noting the number of hours five or more seeds were first and last seen on the surface of the naturally dejected feces. He considered 14–85 hours a rapid rate and 62–134 hours a normal rate.

Alvarez (3) used glass beads in determining the rate of passage of food residues through man and found on an average 75 per cent of the beads were passed in 96 hours.

Cugnini (4), cited by Burnett (2), placed powdered Brazil nuts in the food of horses in 33 tests. The first appearance of the marker varied between 15 and 24 hours and was last seen from 74 to 195 hours.

Reed, Huffman and Addington (5) obtained the rate of passage of Sudan III fed to four heifers. The first and last appearance of the dye was determined from the color of the filtered ether extract of the feces. The first appearance was from 12 hours and 45 minutes to 16 hours. The last appearance was from 47 to 51 hours.

Fish (6) fed Sudan III in butter to a cow and found that the dye first appeared in the feces 16 to 17 hours after feeding and the last appeared from 48 to 92 hours.

Ewing and Smith (7) fed rubber discs to steers as markers. Some of

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the discs were passed in from 12 to 60 hours and some were not passed at all. These investigators later adopted the slaughter method.

Mitchell, Hamilton and Kick (8) suggest, after feeding iron oxide to steers, that the rate of feeding may be an important factor in determining the time of passage of food through the digestive tract. In other words, the food consumed clears the tract of residues from preceding meals in proportion to its mass.

The reviewed literature reveals very few systematic studies of the rate of passage of inert materials through the digestive tract of the bovine. It was with this point in view that the experiments reported in this paper were carried out.

EXPERIMENTAL

In order to measure the rate of passage of inert materials through the digestive tract it is necessary to feed some substance which can be recovered from the feces. It is necessary to feed something which the animal does not digest nor absorb and which does not affect the digestive system. Furthermore, if solid materials are fed, consideration must be taken of the fact that the bovine regurgitates and remasticates a considerable portion of the food consumed. This fact eliminates the use of glass beads, seeds, etc. Iron oxide and rubber discs were used in this investigation.

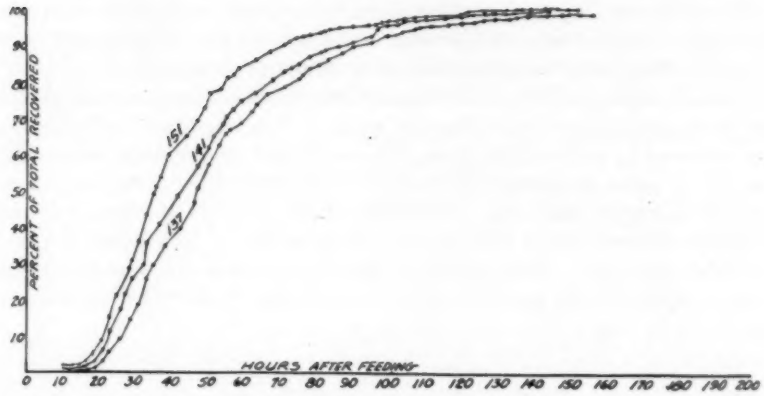
In all of the graphs in this report the total amount of the test materials recovered at the end of the experiment was called 100 per cent regardless of the amount fed. Any point on the graph represents the percentage of the total test material recovered which had been excreted at that particular time. In other words, in Graph I, 40 per cent of the total iron recovered had been accounted for after 42 hours for animal 137. In this manner the curves representing the rate of excretion of each animal can be compared with each other on a similar basis.

EXPERIMENT I

Iron Oxide

One hundred grams of the iron oxide (technical grade 83.1% Fe_2O_3) sufficiently fine to pass through a 100 mesh sieve was fed with the feed of silage to each of three healthy pure bred Holstein cows on the morning the experiment was begun. Constant watch was then kept for 215 hours. Each passage of feces was collected and the time of defecation noted. Each passage was weighed and the iron content of each sample determined by the method of Pincussen and Roman (9). The cows were exercised from 20 to 30 minutes each day during the experiment.

Complete data obtained from each animal were omitted due to the large amount of space required to submit them. Table I gives information relative to each cow and a summary of the results. The rate of excretion of the iron oxide is shown in Graph I.



GRAPH I. SHOWING RATE OF EXCRETION IN HOURS OF IRON OXIDE AS PERCENTAGE OF THE TOTAL RECOVERED FOR EXPERIMENT I.

The results show that the first iron excreted appeared in from 9 hours and 55 minutes to 13 hours and 20 minutes after feeding; that the high point of excretion was 33 hours and 10 minutes for all three animals; that the end points of excretion varied from 143 hours and 10 minutes to 156 hours and 5 minutes. The high point was considered that point at which the greatest amount of test material was excreted per kilo of feces. It was somewhat difficult to tell exactly the end point of excretion since the percentage of iron in the feces may come down to the basal percentage and then rise again slightly for a few passages. The point at which the percentage of iron in the feces did not rise again above the basal value was considered the end point.

Only 82.4 to 88.7 per cent of the iron oxide fed was accounted for. The method of analysis was checked for its accuracy without disclosing any discrepancy sufficiently great to account for the loss.

EXPERIMENT II

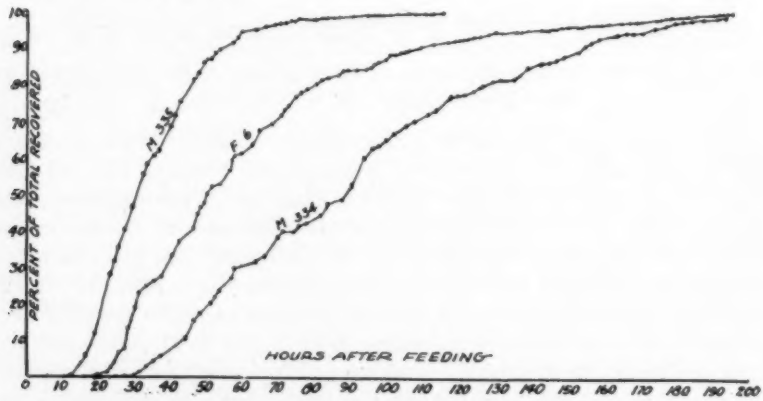
Rubber Rings

Rubber rings were cut about 1.5 to 2.0 millimeters in thickness from heavy walled white rubber tubing one-half inch in diameter with a wall one-eighth inch in thickness.

Three healthy grade Holstein cows were fed 1,000 rubber rings mixed in the silage portion of the ration. Those remaining in the manger after the silage had been consumed were mixed with a little grain so that all of the rings were finally consumed by the animals. One thousand rings occupied about one quart in volume. Constant watch was then kept of the animals. Records were kept of the daily food and water consumption.

The water consumption was determined by means of water meters attached to the drinking cups. The animals were kept on the experiment for 200 hours. They were exercised from 20 to 30 minutes each day.

Each passage of feces was weighed, the time noted, and the hardness or softness determined by mechanical means. The consistency of feces was determined in order to ascertain whether or not the animals remained in about the same physiological condition throughout the experiment insofar as the digestive tract was concerned. Each passage of feces was then washed on a one-eighth mesh screen with a stream of water from a hose to recover the rings. Data pertaining to the animals and a summary of the results obtained are shown in table 1. The rate of excretion of the rings is shown in Graph II.



GRAPH II. SHOWING RATE OF EXCRETION OF RUBBER RINGS AS PERCENTAGE OF THE TOTAL RECOVERED FOR EXPERIMENT II.

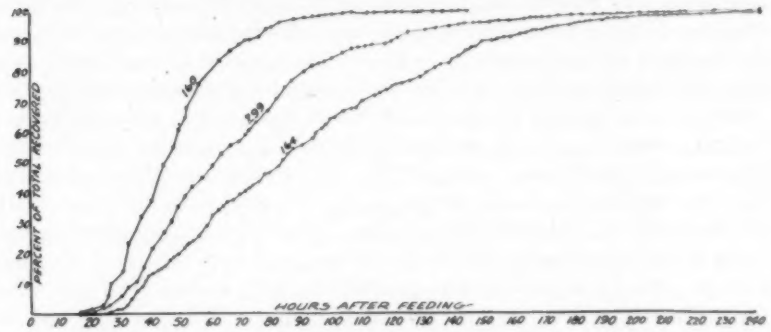
Not all of the rubber rings were recovered except in the case of animal M 335. The first appearance in the feces was from 10 hours and 45 minutes to 18 hours and 7 minutes. The high point of excretion was from 23 hours and 12 minutes to 33 hours and 15 minutes. The end point of excretion could be determined for only one animal, M 335, which excreted the last ring at 114 hours and 35 minutes. The rings obtained from animals M 334 and F 6 along toward the latter part of the experiment were considerably cut up. It was necessary to piece the parts together in counting the rings. This of course introduced some error. The consistency of the feces of the animals varied but little from day to day during the progress of the experiment.

EXPERIMENT III

Rubber Rings

The second experiment using rubber rings was practically a duplicate of the first one except that no silage was included in the feeds. The rings

were fed in the grain mixture. The animals used were three healthy pure-bred cows, kept under constant watch for 215 hours after the rings were fed. Information and data concerning the animals of this experiment are shown in table 1. The rate of excretion of the rings is shown in Graph III.



GRAPH III. SHOWING RATE OF EXCRETION OF RUBBER RINGS AS PERCENTAGE OF THE TOTAL RECOVERED FOR EXPERIMENT III.

All of the rings were not collected from two of the animals. More rings were supposedly recovered from animal 164 than were fed. This discrepancy is due to the error in estimating the number of rings from the pieces or fragments.

The feces of these animals were collected for 72 hours after the 215 hours' period. Constant watch was not kept during this period but the feces were washed about every 12 hours to obtain the rings. The first appearance of the rings was from 17 hours to 19 hours and 30 minutes. The high point of excretion was from 37 hours and 15 minutes to 60 hours and and 30 minutes. The end point of excretion was 141 hours and 52 minutes for animal 164, although one-fourth of a ring was excreted between 226 and 241 hours. It is difficult to set an end point for the other two animals since 100 per cent of the rings was not recovered. However, cow 299 excreted only four and two-thirds rings between 193 hours and 52 minutes and 241 hours, after which none were excreted up to 287 hours when the experiment ended. Animal 160 excreted only three rings between 214 hours and 30 minutes and 287 hours, although only 83 per cent of the rings had been accounted for. The curves showing the rate of excretion of the rubber rings show considerable variation. Animal 160 was exceedingly slow.

The consistency of the feces varied but little during the progress of the experiment. The animals on this experiment which received no silage consumed considerably more water than the animals on the previous experiment which received silage.

DISCUSSION OF RESULTS

The use of iron oxide might be criticized because of its possible astringent effect although it is supposed to be practically insoluble in the digestive tract. Hoelzel (1) was inclined to believe that iron oxide acted as an astringent. In Experiment I of the present paper 400 rubber rings fed to the cows 48 hours after the iron oxide had been fed was excreted at almost the identical rate at which the iron had been excreted for each cow. The fact that not all the iron oxide fed was recovered is not surprising in view of the work of Hoelzel (1), who found that in animals with which he worked certain materials might be retained in parts of the digestive tract for considerable periods of time. Gallup (10), and Heller, Breedlove and Likely (11) were unable to recover 100 per cent of the iron oxide fed to rats when the Bergeim (12) method for studying digestibility was used. Furthermore, in this investigation not all the rubber rings were recovered, although a better percentage recovery was secured with rubber rings than iron, after a considerable period of collection which demonstrated that they had become lodged or settled in some part of the digestive tract. These results indicate that the Bergeim (12) method of investigating digestibility where iron oxide is used would not be accurate for the bovine.

It is interesting to note that animal 151, in which the iron oxide first appeared in the feces in 9 hours and 55 minutes after being fed, showed the fastest rate of excretion. Animal 141, in which the iron oxide appeared in 10 hours and 35 minutes, showed the medium rate, while animal 137, in which the iron oxide first appeared in 13 hours and 20 minutes, showed the slowest rate of excretion In Experiment II, M 335, the animal which first started to excrete the rings and from which the most rings were recovered, reached the high point of excretion first and showed the fastest rate of excretion. The other two animals follow in a like manner in these respects. In Experiment III, the animal which first started to excrete the rings, also reached the high point of excretion first, and from which the most rings were recovered also showed the fastest rate of excretion. Animal 160 proved to be an exception in one respect in that she excreted the first ring in 17 hours which was the same as Animal 164, but showed the slowest rate of excretion. However, 160 did not excrete any more rings until 5 hours later when she excreted four, although she made two defecations in the mean time. By 30 hours she had excreted only 13 rings while animal 164 had excreted 104.

It appears from the results of this investigation that under comparable conditions those animals which first excrete the test material and reach the high point of excretion first, have the fastest rate of excretion. A study of the graphs indicates that the feeding of a test material and collection for 60 hours afterwards gives a comparable method for studying the rate of passage of inert materials through the digestive tract. It is difficult to measure

the complete lag, probably due to the fact that the test material becomes lodged or settles in some part of the digestive tract and may not be excreted for a considerable period of time.

These results indicate that feeds fed to bovine may exert some influence even up to 150 hours after feeding. They also show that in experiments with the bovine where the feeds are changed from one régime to another the lag must be taken in consideration in the interpretation of results.

The animals used in this investigation were on a medium plane of nutrition. It would be interesting to study the effect on the rate of passage of inert materials in animals on a high and a low plane of nutrition. It would likewise be interesting to study the rate of passage from the abomasum, excluding the rumen, reticulum, and omasum. However, due to the expense involved in keeping constant watch these points have not been investigated.

SUMMARY AND CONCLUSIONS

1. The rate of passage of iron oxide through the digestive tract was studied in three cows. The rate of passage of rubber rings was studied in six cows.

2. The iron oxide first appeared in the feces in from 9 hours and 55 minutes to 13 hours and 20 minutes. The rubber rings first appeared in from 10 hours and 45 minutes to 19 hours and 30 minutes.

3. The high point of excretion of the iron oxide was 33 hours and 10 minutes. The high point of excretion for the rubber rings was from 23 hours and 12 minutes to 60 hours and 30 minutes.

4. The lag varied from 114 hours and 35 minutes to 156 hours and 5 minutes for the iron oxide and from 141 hours and 52 minutes to approximately 215 hours for the rubber rings.

5. It appears that those animals under comparable conditions which first excrete the test material and first reach the high point of excretion show the fastest rate of excretion.

6. The feeding of a test material, collection and examination of the feces for 60 hours afterwards gives a comparable method for investigating the rate of passage of inert material.

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TABLE 1

Information relative to the animals used and a summary of the results obtained

ANIMAL	AGE	WEIGHT	AVERAGE DAILY							RESULTS			
			Grain Fed	Hay Fed	Corn Silage Fed	Water Consumption	Milk Production	Defecations	Feces	First Appearance	High Point	Last Appearance	Total Recovered
No.	*yrs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*No.*	*gms.*	*hrs. & min.*	*hrs. & min.*	*hrs. & min.*	*per cent*
Experiment I. 100 grams iron oxide fed													
151	8	1450	4	12.0*	24	.....	0.0	9.6	23609	9:55	33:10	152:55	84.7
141	8½	1450	4	12.0*	24	.....	0.0	9.0	25254	10:35	33:10	143:10	82.4
137	9	1600	4	12.0*	24	.....	8.3	9.0	21518	13:20	33:10	156:05	88.7
Experiment II. 1,000 rubber rings fed													
M 334	5	1311	10	9.0**	30	47	15.4	9.1	19661	18:07	33:15		95.0
M 335	5	1390	11	10.0**	30	61	1.8	11.7	25596	10:45	23:12	114:35	99.9
F 6	3	1062	15	8.0**	20	79	40.7	12.6	22820	15:58	28:17	-	96.5
Experiment III. 1,000 rubber rings fed													
229	5	1319	6	18.5***	0	80	14.0	8.8	22877	19:30	40:35		90.8
164	3	1184	8	17.9***	0	82	19.7	8.4	21735	17:00	37:15	141:52	100.5
160	3	1138	10	17.5***	0	76.2	15.0	13.2	25754	17:00	60:30		83.0

* Coarsely cut mixed hay.

** Coarsely cut timothy.

*** Alfalfa.

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STUDIES ON WHIPPING CREAM. II*

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In an earlier paper[1] under this title studies were presented on whipping cream in which the factors discussed were: the fat content of the cream, the time and temperature of aging, the reaction of the cream as measured in terms of titratable acidity, and the effect of added salts. In this paper further data on the effect of reaction are given together with data on the following factors:

1. Temperature of Cream Separation.
2. Cream Pasteurization.
   a. Temperatures.
   b. Cooling after Pasteurization.
3. Homogenization of the Cream.
   a. Temperatures.
   b. Pressures.

The properties of the whipped cream studied were the same as before, namely: whipping time, stiffness of the whipped cream, overrun, and drainage.

The apparatus and procedures were the same as described in the first paper[1] so a brief summary will be sufficient at this time. The cream whipping was performed in a room having a temperature range of 38 to 44° F. (3.3 to 6.6° C.). The turbine whipper was driven by a motor of sufficient power to maintain a constant speed. The load on the motor and its rate of increase were noted at regular intervals from the gear arrangement and scale as described previously.[1] The terms used in the accompanying tables have been defined. "Average torque increase per second" is the figure obtained by dividing the increase in torque as expressed in grams by the number of seconds that the cream was whipped. As is evident from the curves this value varies as the whipping progresses, but the average value is useful for purposes of comparison. For example, if one sample of cream whipped through a torque of 88 grams in 220 seconds and another through

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[1] Hugh L. Templeton and H. H. Sommer. Studies on whipping cream. JOUR. DAIRY SCIENCE, 16: 329–345. 1933.

a torque of 116 grams in 290 seconds, the "average torque increase per second" would be the same, namely 0.400.

The overrun was determined by weighing a definite volume of cream and an equal volume of the whipped cream, care being taken to prevent undue agitation of the whipped cream.

The data presented in this paper may be taken as typical of the results obtained in the study of each of the factors enumerated. Space limitations do not permit the presentation of the complete whipping record of each sample given in the tables; only the "average torque increase per second" is given. A few curves are included to illustrate typical whipping records and the corresponding samples in the tables are indicated. Similar curves were drawn for each of the samples in the tables and it was found that the "average torque increase per second" is a reliable basis for comparison.

The reaction of cream to be used for whipping has received considerable attention. Apparently it is quite common for practical dairymen to consider "ripening" of cream as a possible remedy for unsatisfactory whipping. Accordingly the effect of reaction was studied comparing normal, sweet cream with cream that had been acidified by the addition of acids. The acidity of the cream is given in terms of titratable acidity calculated as lactic acid rather than in terms of hydrogen-ion concentration, because the average dairy plant does not have the equipment needed for the latter determination. The titratable acidity of both the cream and the serum are given. The acidity of the serum was determined by titrating a 9 cc. portion of the drainage from the first whipping of the cream.

The results of this study on the effect of reaction are presented in table 1 and figure 1. The table is divided into sections according to the fat content of the cream used, the results given in each section having been obtained on different batches of cream. Section "A" of the table is a comparison of the effects of added lactic and citric acids. It will be noted that the whipping time decreases as the titratable acidity increases and that the addition of citric acid gives more pronounced effects than lactic acid. The cream containing the more acid was rather viscous and the whipped cream was rather soft, but it held the serum very tenaciously as shown by the length of time necessary for drainage and the smaller amounts of serum that drained from the cream.

Section "B" gives the data for successive increases in the amount of added lactic acid. The results show the decrease in whipping time with increasing acidity. The sudden drop in the value for the overrun was expected from the viscosity of the cream and the fact that the whipped cream did not whip up, but remained as a rather soggy mass in the bottom of the dish when the torque indicated the maximum stiffness attainable with apparatus. It was noted from time to time in the different experimental whips that the cream with the most noticeable increase in viscosity did not

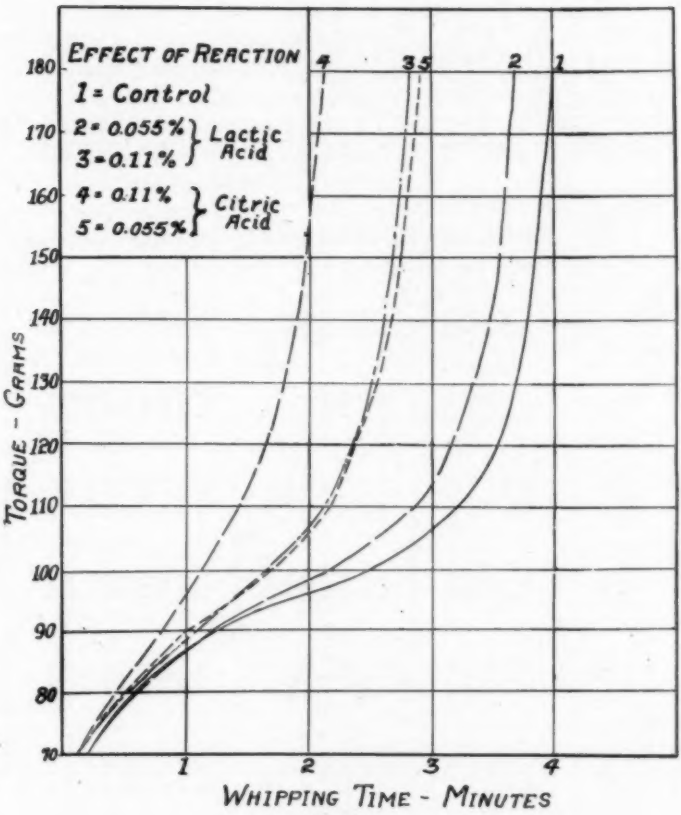

FIG. 1

whip as well as cream that was less viscous. It has also been noted in connection with the increase in acidity that there is a point where the overrun drops sharply. This usually occurs when the titratable acidity is about 0.30 per cent calculated as lactic acid. This high acid cream shows very little drainage and the fat content of the serum is very low.

Section "C" compares the effect of the addition of the acid before and after pasteurization. It is very interesting to note that with the increase in acidity above 0.20 per cent that the cream acidified before pasteurization whipped much more rapidly than that to which the acid was added after the cream had been cooled to 15° C. Below 0.20 per cent acid the creams whipped at about the same rate. The overrun was very uniform for the entire series. The values obtained in the study of the drainage are quite in agreement with those presented in the other sections of this table.

While the above results show that an increased acidity in general im-

TABLE 1

Effect of reaction on whipping quality

SAMPLE NO.	REAGENT ADDED		TITRATABLE ACID		WHIP TIME SEC.	AVERAGE TORQUE INC./SEC.	OVER-RUN %	DRAIN			
	Kind	%	Cream %	Serum %				Time secs.	Weight grams	Fat %	Fat loss
					"A" 32% butterfat						
1	Control		0.11	0.13	239	0.551	165	763	42	4.43	1.87
2	Lactic Acid	0.055	0.15	0.19	222	0.588	169	476	37	6.10	2.27
3	" "	0.11	0.19	0.21	170	0.778	173	1144	27	5.15	1.37
4	Citric Acid	0.11	0.26	0.26	128	1.018	162	1565	23	3.75	0.85
5	" "	0.055	0.17	0.18	175	0.752	174	533	41	5.55	2.21
					"B" 30% butterfat						
6	Control		0.11	0.13	346	0.373	166	351	57	3.65	2.10
7	Lactic Acid	0.011	0.13	0.14	394	0.327	160	369	56	3.63	2.01
8	" "	0.033	0.14	0.16	336	0.370	166	454	57	4.05	2.31
9	" "	0.055	0.16	0.19	324	0.389	170	415	56	5.05	2.82
10	" "	0.088	0.19	0.22	294	0.422	171	415	47	5.23	2.43
11	" "	0.11	0.21	0.22	259	0.493	171	579	31	4.40	1.37
12	" "	0.22	0.31	0.32	186	0.711	141	1465	32	0.43	0.14
					"C" 32½% butterfat						
13	Control		0.11	0.14	172	0.707	162	487	45	5.45	2.43
14	Lactic Acid	0.055	0.15	0.20	154	0.813	165	617	40	5.28	2.11
15	" "	0.055	0.16	0.20	156	0.795	168	393	51	5.43	2.72
16	" "	0.11	0.21	0.22	133	0.939	168	1318	21	3.63	0.82
17	" "	0.11	0.20	0.25	158	0.792	167	471	51	5.50	2.75
18	Citric Acid	0.055	0.17	0.21	157	0.778	165	496	44	5.55	2.41
19	" "	0.055	0.19	0.21	149	0.813	165	334	49	6.03	2.93
20	" "	0.11	0.23	0.26	104	1.199	162	1354	20	3.45	0.68
21	" "	0.11	0.24	0.31	141	0.877	168	322	52	4.50	2.27

Acid added to samples Nos. 14, 16, 18, 20 before the cream was pasteurized; Acid added to samples Nos. 15, 17, 19, 21 after the cream had been pasteurized and cooled to 15° C.

proves the whipping of the cream, it does not follow that the beneficial effect of aging is due to an increase in acidity, since it is usually true that there is no measurable increase in acidity on aging. These results on the effect of reaction are in quite satisfactory agreement with the data presented by Babcock.[2] From work that will be presented later it would seem that one of the more essential features of aging is the solidification of the fat globules and the formation of clusters.

There are a number of factors involved in the production of satisfactory whipping cream that might be grouped under the general heading of plant procedures. The first of these to be presented is the temperature at which the cream is separated. The results of this study are given in table 2 and figure 2. The table has three sections and it should be noted that the cream used in the first two sections was from winter milk while the last series was

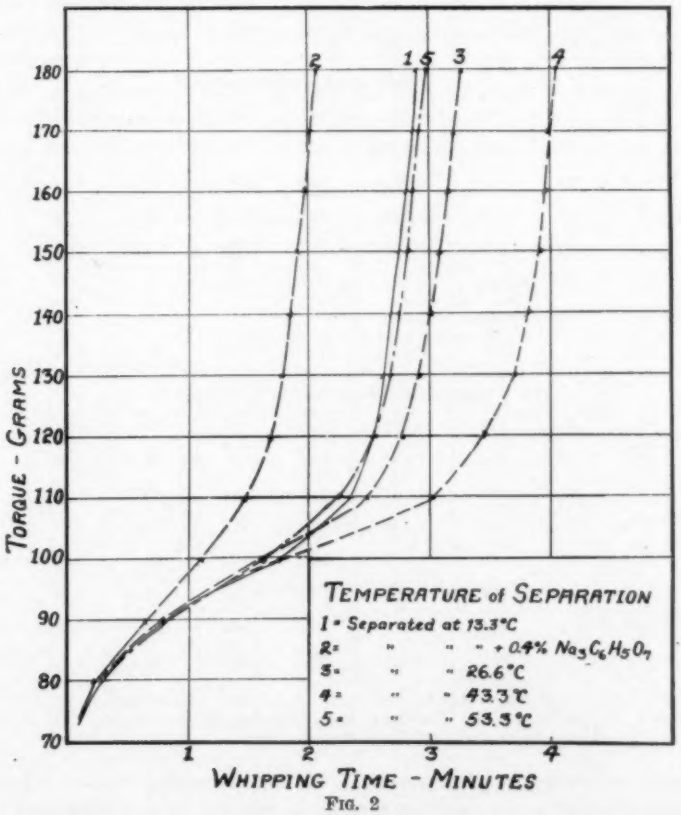

FIG. 2

[2] S. J. Babcock. The whipping quality of cream. U. S. Dept. Agr. Bulletin No. 1075. July 1922.

TABLE 2

Temperature of cream separation

CREAM			WHIP. TIME SEC.	AVERAGE TORQUE INC./SEC.	OVER-RUN %	DRAIN			
Sep. °F.	Temp. °C.	Fat %				Time sec.	Weight gms.	Fat %	Fat loss
I. Cream standardized to 32½ or 33% butterfat									
56	13.3	58	176	0.727	161	552	48	1.90	0.91
56*	13.3	58	124	1.016	154	373	54	3.00	1.61
80	26.6	55	196	0.641	161	499	47	4.48	2.12
80*	26.6	55	172	0.723	153	406	60	4.65	2.80
110	43.3	53	244	0.483	160	528	46	7.93	3.71
110*	43.3	53	163	0.768	151	527	55	6.48	3.59
128	53.3	49	177	0.711	160	415	51	10.35	5.24
128*	53.3	49	171	0.734	157	276	60	7.58	4.58
II. Cream standardized to 30% butterfat									
60	15.6	54	209	0.581	165	476	51	3.63	1.86
60†	15.6	54	180	0.645	157	421	54	2.60	1.40
85	29.4	56	196	0.605	161	587	50	4.50	2.24
85†	29.4	56	216	0.548	155	651	53	2.95	1.55
105	40.5	55	304	0.400	162	396	59	7.15	4.23
105†	40.5	55	168	0.724	161	436	61	9.55	5.90
126	52.2	51	440	0.266	170	441	59	6.15	3.60
126†	52.2	51	286	0.431	171	470	66	7.83	5.18
III. Cream standardized to 30–31% butterfat									
72	22.2	46	449	0.263	136	387	37	3.40	1.22
72‡	22.2	46	396	0.284	122	324	40	2.40	0.95
100	37.8	36	623	0.097	130	94	62	9.20	6.22
100‡	37.8	36	425	0.283	143	229	51	5.68	3.01
125	51.6	34	619	0.105	132	132	61	9.58	6.41
125‡	51.6	34	494	0.200	135	195	55	6.30	3.49
150	65.5	31	424	0.289	150	371	44	7.25	3.25
150‡	65.5	31	296	0.398	157	332	56	6.85	3.79

* 0.40% sodium citrate added to these samples before pasteurizing.
† 0.20% sodium citrate added to these samples before pasteurization.
‡ 0.40% sodium citrate added to these samples.

from milk produced in midsummer. This also accounts for the difference in temperature at which the first sample in each series was separated. All the creams were standardized with skimmilk collected at the same time as

the cream. The milk was heated in a tubular heater and the temperature taken of the milk as it entered the separator. The fat content of the creams separated at the different temperatures is given to show the decrease in fat with the increasing temperature. Sodium citrate in the amounts indicated was added to half of the samples in order to compare its effectiveness on the different creams. It should be noted that in all but one instance the addition of the citrate decreased the whipping time. In the first two series there are no significant differences in the overrun. It is evident that in order to minimize the fat losses in the serum it is wise to separate the cream at a low temperature. From the first and third series it is shown that the whipping time increases with the temperature and then decreases again. It is possible that if there had been a higher temperature used in the second series that the whipping time would have decreased at the higher temperature. No explanation can be offered at this time for the variations occurring in the third series especially in regard to the low overrun values.

The temperature at which the cream was pasteurized was found to have very little effect upon the whippability. As shown in table 3 and figure 3 there is a slight improvement in the whipping time and the length of time

TABLE 3

Temperature of pasteurization

PAST. TEM. °C.	WHIP. TIME SEC.	AVERAGE TORQUE INC./SEC.	OVER-RUN %	DRAIN			
				Time sec.	Weight gms.	Fat %	Fat loss
I. Cream containing 36% butterfat							
60–61.5	128	1.027	153	829	26	4.00	1.00
60–61.5*	141	0.898	138	616	30	2.95	0.87
65–66	130	1.013	152	600	23	7.50	1.67
65–66*	150	0.843	135	887	28	4.75	1.33
70–71	116	1.124	152	792	22	9.25	2.01
70–71*	135	0.950	139	509	28	6.35	1.61
II. Cream containing 29½% butterfat							
60–61.5	371	0.320	155	458	40	2.50	1.00
60–61.5*	319	0.397	153	553	40	2.13	0.84
65–66	375	0.335	156	567	38	4.35	1.68
65–66*	255	0.526	160	413	47	4.23	2.12
70–71	340	0.358	156	610	40	5.00	1.97
70–71*	247	0.513	159	454	46	4.63	2.08

* 0.40% sodium citrate added to these samples.

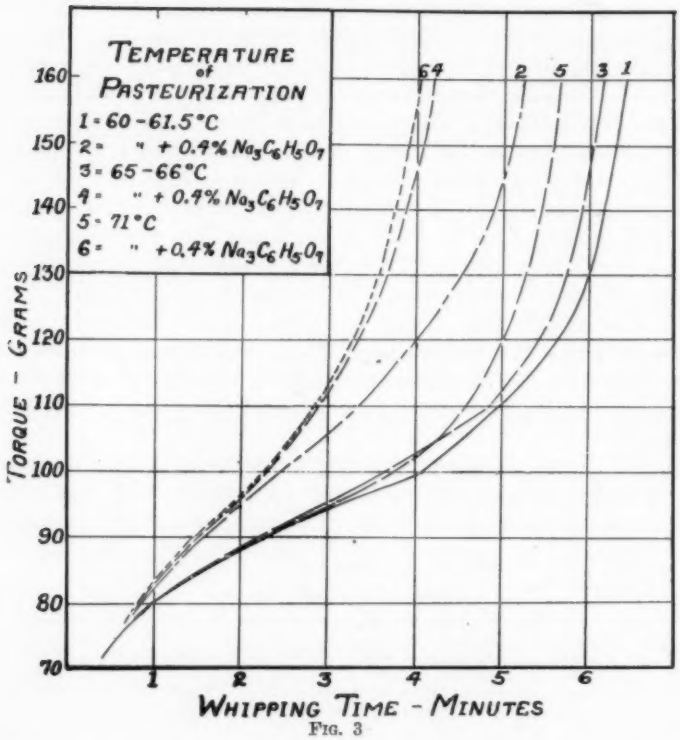

FIG. 3

that the serum remains in the whipped cream when the cream was pasteurized at 70–71° C. This difference can hardly be considered as sufficient to warrant the use of the higher temperature as there is the possibility of the cream getting a slight scorched flavor. It should be noted that all the samples were kept at the temperatures indicated for the full thirty minutes. In the first section of this table the fat content of the cream was too high for the addition of sodium citrate to have any beneficial effect as regards decreasing the whipping time.

After the cream has been pasteurized it is very essential that it be cooled to 15° C. or below before it is put into storage. An example of the effect of poor cooling is given in table 4. In this instance the cream was cooled to 22° C. before it was bottled and placed in the storage room at 6.6° C. While the fat content (31 per cent) of this cream was high enough to warrant a satisfactory whipping quality, and as shown in the preceding table the temperature of pasteurization has little effect upon the whippability, this cream did not whip. As shown in the second part of the table the whipping quality improved after storage for 70 hours. This combination

TABLE 4

Temperature of pasteurization. Effect of poor cooling after pasteurization

PASTEURIZATION TEMP. °C.	WHIP. TIME SEC.	AVERAGE TORQUE INCREASE PER SEC.	OVERRUN %	DRAIN			
				Time sec.	Weight gms.	Fat %	Fat loss
I. Less than 48 hours' storage at 44° F. Cream containing 31% butterfat							
58–61	360	0.115	134	22	85	17.00	14.79
58–61*	390	0.130	133	37	71	11.25	8.03
64–66	360	0.112	135	26	95	21.00	19.93
64–66*	360	0.140	133	35	64	15.25	10.37
69–71	360	0.119	134	24	83	21.50	18.87
69–71*	318	0.293	144	220	43	9.25	4.04
II. After 70 hours' storage. Cream containing 31% butterfat							
58–61	330	0.390	164	1058	24	3.00	0.71
58–61*	210	0.619	168	839	33	3.25	1.07
64–66	263	0.544	170	668	27	7.00	2.05
64–66*	233	0.531	155	825	29	4.00	1.16
69–71	320	0.401	153	599	17	4.25	0.70
69–71*	203	0.643	154	688	32	5.00	1.63
III. Cream cooled to 2° C. and whipped after eight hours at that temperature. Cream containing 32% butterfat							
No. 1	132	0.808	168	326	45	8.50	3.66
No. 2†	142	0.880	171	293	40	7.70	3.08
No. 3‡	142	0.822	172	483	39	7.20	2.81

* 0.40 % sodium citrate added to these samples.
† 0.055% lactic acid added before pasteurization.
‡ 0.046% citric acid added before pasteurization.

of circumstances certainly indicates that it is quite necessary to cool cream intended for sale as whipping cream to temperature sufficiently low to avoid difficulties. In the regular routine preparation of samples, the cream was cooled to at least 15° C. before it was bottled. In the third section of this table a few data are presented to show the effect of thorough cooling at low temperatures on the whippability of cream. The slight difference in the fat content cannot be given as the reason for the better whipping after such a short period of storage.

It is well known that the whipping quality of ice cream mixes is greatly improved by homogenization and there have been claims made by the manufacturers of homogenizers that whipping cream would whip much more satisfactorily if homogenized at low pressures. The results of the study

TABLE 5

Homogenization

PRESSURE POUNDS	WHIPPING TIME SEC.	AVERAGE TORQUE INC./SEC.	OVERRUN %	DRAIN			
				Time sec.	Weight gms.	Fat %	Fat loss
I. Above 50° C. Cream containing 30½% butterfat.							
Con.	263	0.469	174	509	51	4.50	2.31
Con.*	183	0.691	167	474	54	3.58	1.95
100	408	0.178	167	166	88	15.75	14.32
100*	340	0.368	175	391	65	9.95	6.26
200	390	0.120	160	54	131	22.25	29.22
200*	402	0.223	171	191	90	16.00	14.74
300	390	0.121	162	47	131	24.25	32.02
300*	383	0.182	180	190	111	19.88	22.54
400	375	0.139	170	61	126	23.50	29.72
400*	390	0.157	176	81	122	22.38	27.48
II. 30 to 37° C. Cream containing 30½% butterfat.							
Con.	199	0.627	172	476	40	5.10	2.03
Con.†	172	0.738	168	399	46	5.00	2.32
100	228	0.548	170	486	44	5.45	2.40
100†	158	0.787	171	555	49	5.15	2.54
200	338	0.366	170	637	49	6.85	3.32
200†	233	0.534	171	627	53	5.90	3.13
300	390	0.140	148	101	100	23.75	23.72
300†	331	0.357	173	689	64	10.75	6.90
400	420	0.123	140	75	106	24.38	25.83
400†	413	0.256	169	324	81	16.38	13.56
III. Below 28° C. Cream containing 32% butterfat							
Con.	146	0.902	162	659	42	4.90	2.04
Con.‡	104	1.262	158	959	42	4.45	1.85
50	140	0.959	160	477	44	4.90	2.15
50‡	132	0.987	157	482	48	6.65	3.22
100	169	0.772	161	633	47	5.88	2.78
100‡	155	0.841	153	511	51	7.08	3.59
200	209	0.622	161	605	47	8.83	4.15
200‡	179	0.730	156	649	53	9.23	4.85
400	266	0.487	160	587	52	11.50	5.94
400‡	202	0.639	157	597	55	10.63	5.87

* 0.40% of sodium citrate added to these samples immediately after homogenization.
† 0.30% sodium citrate added to these samples after homogenizing.
‡ 0.30% sodium citrate added to these samples of cream before pasteurization and homogenizing.

of the homogenization of whipping cream are given in table 5 and figure 4. The cream was homogenized with a two stage machine but using only one stage. A special pressure gauge was used, graduated from 0 to 400 pounds. In the first section of table 5 homogenization was started as soon as the cream was pasteurized, but due to trouble in the adjustment the temperature dropped about 11° C. before all the samples had been obtained. It was found that an attempt to portray the results in the same manner as before gave a number of lines that were almost identical. In view of the

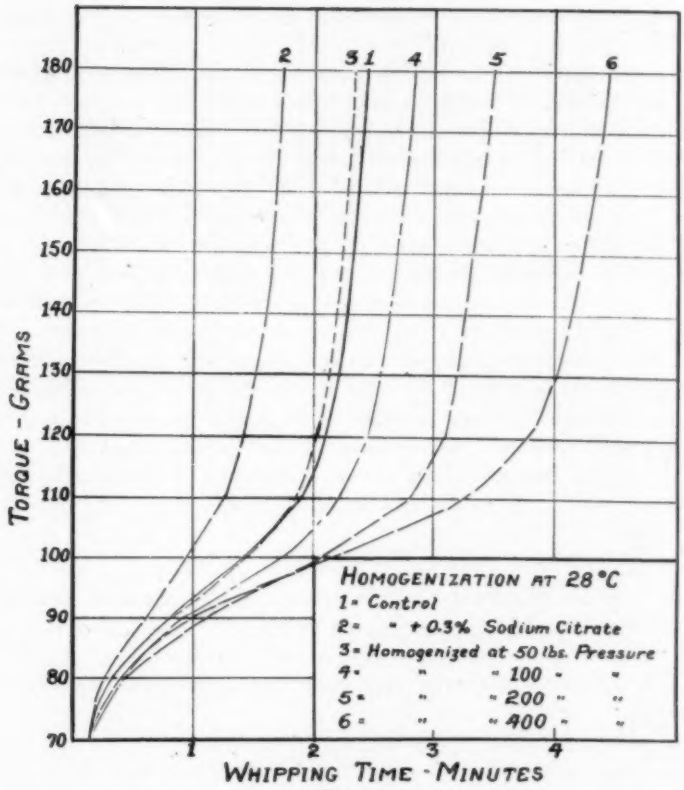

FIG. 4

results obtained in the other series of experiments on the effect of homogenization the failure of the sodium citrate to decrease the whipping time at the higher pressures was unexpected and no explanation can be offered at this time.

As compared with the control samples, homogenization had a slight beneficial effect upon the overrun but the slight increase was more than counterbalanced by the longer whipping time and the excessive drainage which had a high fat content. In this connection it is interesting to note the effect of the addition of sodium citrate in increasing the drainage time, decreasing the amount of drainage and also the fat content.

The second section of table 5 is not presented in chart form. In the table it should be noted that the addition of sodium citrate to the samples usually decreased the whipping time, this decrease ranged from 7.3 per cent at 300 pounds pressure to about 31 per cent at 100 and 200 pounds. The longer whipping time for the samples homogenized at 300 and 400 pounds

would lead one to expect the relatively poor overrun with the accompanying high fat loss in the drainage.

The third section of the table is presented in graph form in figure 4. As noted on the table the sodium citrate was added to the cream before pasteurization, and the cream was cooled to 28° C. before homogenization was started. In this series the decrease in the whipping time with the addition of sodium citrate was just the reverse of the preceding being only 5.7 per cent at fifty pounds and 24.0 per cent at 400 pounds. The use of 50 pounds pressure resulted in a fairly satisfactory whipping cream, but unless a producer has the necessary gauges for the homogenizer the problem of regulation is one that will require constant supervision. The overrun of all samples in this series is very uniform, the drain time is also quite uniform among the homogenized samples. In accordance with the results presented in the preceding sections of the table the fat content of the drain and the fat losses increase with the increase in the pressure applied to the cream during homogenization. As a generalization it might be said that the addition of 0.30 per cent of sodium citrate is equivalent to a reduction of 100 pounds pressure in the whipping time of the cream.

SUMMARY

The reaction of normal sweet cream is probably the most satisfactory for the production of a good whipping cream.

If the cream has developed a slight acidity, but not enough to affect the taste it is better to pasteurize it at once rather than neutralize and then pasteurize.

The addition of small amounts of acid (less than 0.03 per cent) does not have an appreciable effect upon the cream. If the titrable acidity calculated as lactic acid is above 0.27 per cent the cream will whip rapidly, but such a cream is eliminated from practical consideration because of its sour taste and the low overrun and soggy appearance of the whipped product. Fat losses in the cream decrease with increasing acidity.

Cream separated at temperatures of 60 to 72° F. (15.5 to 22.2° C.) and 150° F. (65.6° C.) whipped better than creams separated at intermediate temperatures. The lower separating temperatures gave better results as regards the amount of serum lost and its fat content.

The temperature of pasteurization is not an important factor as far as whipping quality is concerned. The usual temperatures are very satisfactory.

Cream intended for whipping must be thoroughly cooled before it is bottled and placed in storage for aging.

Poorly cooled cream showed a marked increase in whippability after storage for more than 70 hours at 44° F. (5.6° C.).

Cream should not be used for whipping until it has aged for 24 hours. If it is necessary to use the cream after a shorter period of aging, the temperature of storage should be as close to 0° C. as possible.

Homogenization of the cream does not have a beneficial effect. If the cream is homogenized it should be done at a low temperature and the pressure should not exceed 100 pounds. It is essential that the gauges be watched continually to avoid changes in pressure. The addition of sodium citrate to the cream before pasteurization decreases the whipping time; the same effect was noted when sodium citrate was added immediately after homogenization. Fat losses in the drainage increase markedly with increasing homogenization pressures.

In conclusion the authors wish to express their thanks to Chas. Pfizer and Co., Inc., who have sponsored the fellowship that has made this study possible.

FACTORS INFLUENCING THE INITIAL INDUCTION PERIOD IN THE OXIDATION OF MILK FAT[1]

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Flavor defects in milk and cream which have been described as "oxidized," "cardboard," "cappy," or "metallic" have been observed for some time in milk and cream which have been exposed to sunlight or contaminated by certain corrosive metals such as copper and some of its alloys. It has been reported by a number of investigators that the change in flavor is caused by oxidation of the milk fat. The active oxidation of a fat is preceded by a period of varying length in which oxygen is not absorbed. This period depends on the chemical constitution of the fat, temperature of holding the fat during processing, storage and exposure to catalysts. This period is known as the induction period and is a measure of the stability of the fat. This paper is concerned with the measurement of the influence of sunlight, certain metals and submaintenance rations of the cow, on the initial induction period in the oxidation of milk fat.

Hammer and Cordes (1) were, apparently, the first to report that exposure of milk to sunlight produced tallowy flavors. The effect was determined organoleptically. This observation was confirmed by Frazier (2) and Tracy and Ruehe (3). Frazier further postulated that the presence of a metal catalyst, resulting from contact with equipment or other sources, would, undoubtedly, assist the catalytic action of daylight. Tracy and Ruehe concluded from their work that "diffuse light is an important factor in the development of tallowy flavors in milk, especially that containing an added copper salt." The influence of sunlight and metals on the induction period were not determined by these investigators. Anderson and Triebold (4) in studying the influence of irradiation of butter on the fat constants and keeping quality of milk fat found that irradiation of a sample of butter reduced the induction period approximately 25 per cent.

Many experiments have been reported where copper and certain other metals corroded by milk are said to have caused "oxidized" flavor in milk (5). The method used in the experiments referred to, however, was organoleptic. No work so far as our search of the literature has been able to disclose, has been reported on the influence of pasteurizing cream in the pres-

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ence of copper and other metals upon the induction period of the milk fat. Briggs (6) and Wright (7) added different metallic salts to butterfat and other fats and determined the influence of such treatment on the induction period. The adding of metalic salts directly to fat, however, is not comparable to pasteurizing the cream in contact with the metal. The greater reduction of the induction period when metallic salts were used, as shown by both Briggs and Wright, bears out this last statement.

EXPERIMENTAL

Briggs (6) and Wright (7) determined the induction period by the oxygen absorption method. Greenbank and Holm (8) used this method in their earliest work and later developed the photochemical method (9). This latter method consists of the observation of the decolorization of methylene blue in pure dry milk fat by means of a photoelectric cell and appropriate accessory apparatus. Greenbank and Holm conclude from their work in developing the method that the rate of reduction of methylene blue in a fat or oil when catalyzed by light served as a measure of the rate of reaction of the initial oxidative processes and that it could be utilized to determine the relative susceptibilities of fats and oils to oxidation. Royce (10) used the method with certain modifications in a study of the "rancidity" of cottonseed oil. The apparatus used in the work reported in this paper (Fig. 1) was essentially like that of Greenbank and Holm (9) with the following modifications.

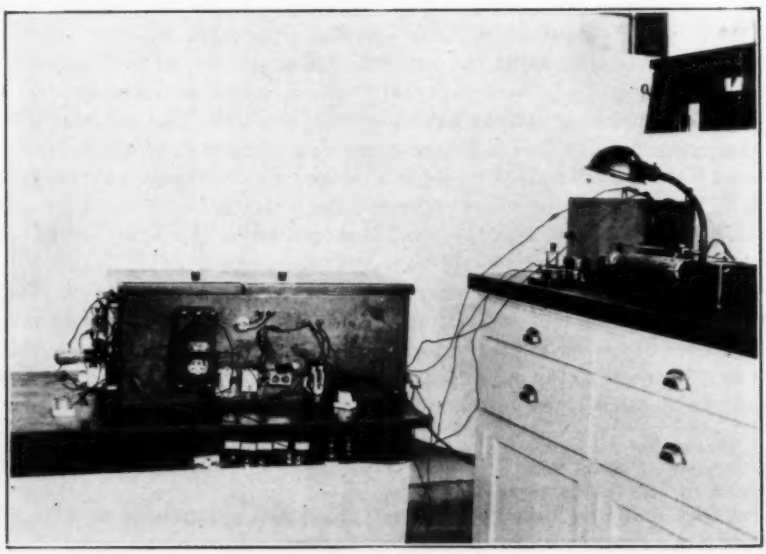


FIG. 1

1. The reaction cell was redesigned in order to permit the constant stirring of the fat during the test (Fig. 2). This latter was found necessary in order to insure uniform fading of the methylene blue.

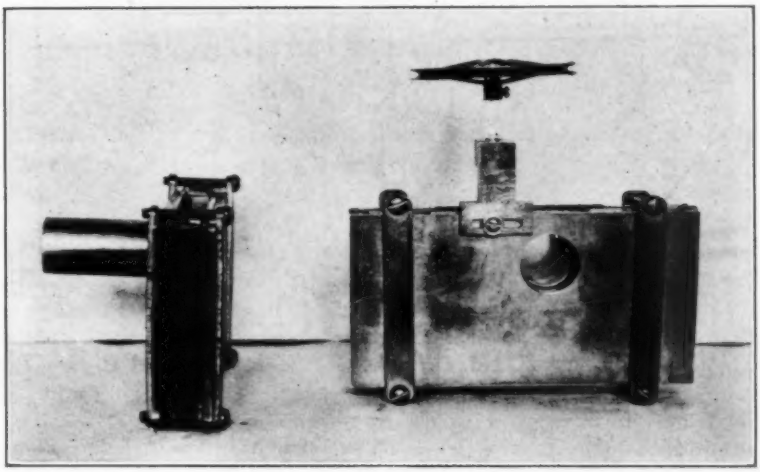


FIG. 2

2. It was found that a 100 watt–110 volt A. C. Mazda lamp could not be used under the operating conditions available. The variations in line voltage caused excessive variations in the intensity of the light, and, hence, in the output of the photo-cell. A constant light intensity is necessary in using a photoelectric cell to detect color changes. A Leeds & Northrup 12–16 volt current regulator and a 50 candle power 12 volt automobile lamp were found to give a constant light source.

3. It was found that the addition of the methylene blue solution permanently changed the color of the fat and that after several hours in the test apparatus the amount of light passing through the fat never equalled that of the pure fat to which methylene blue had not been added. Visual fading of the fat occurred when the amplified output of the cell had increased approximately 1.5 milliamperes. For the conditions described in this experiment an increase of 1.5 milliamperes was arbitrarily chosen as the end-point of the fading of methylene blue. This end-point made the readings comparable when samples of the same fat, treated differently, were compared with each other and when the amount of methylene blue and the distance of the light from the reaction cell were kept constant.

4. A forward-connected photo-electric cell current circuit (Fig. 3) was used in place of the reverse connected circuit used by Greenbank and Holm and by Royce. This circuit is so designed that an increase in light falling upon the cell produces an increased output shown on the milliameter.

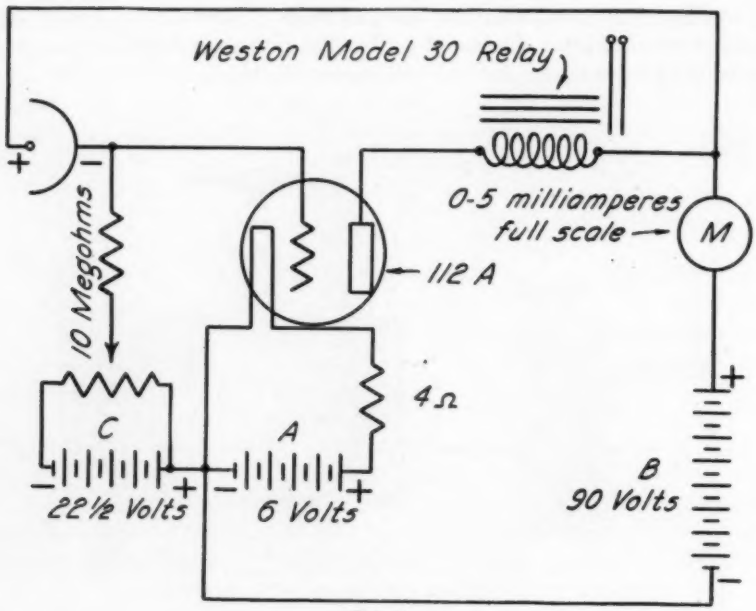


FIG. 3

Preparation of experimental material: In preparing the fat samples for testing, the cream and milk from which it was separated were protected from light, high temperatures and metals corroded by milk, in order that the induction period of the control samples would represent the true susceptibility of the fat to become oxidized. The cream was churned in a dark room in a glass churn. The butter was melted and filtered in a 45° C. oven and then stored at −5° C. and protected from light until the tests were made in the apparatus.

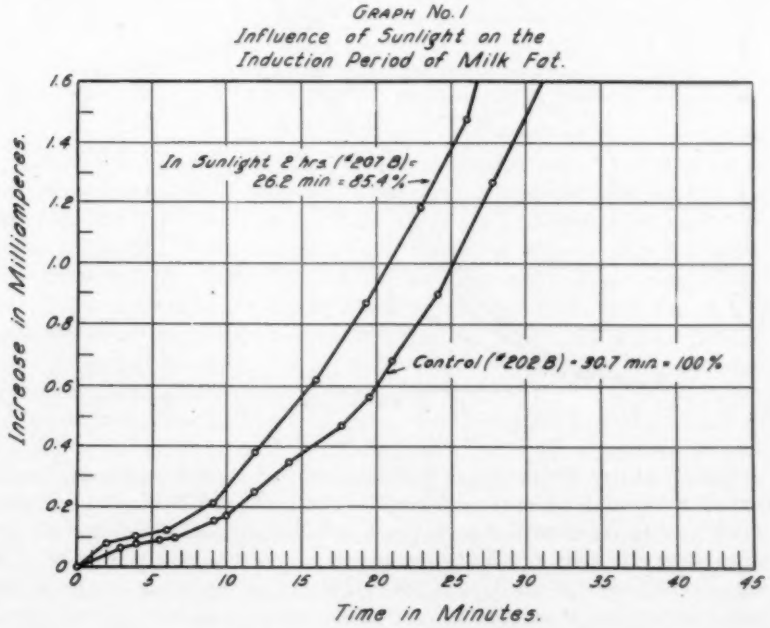
When it was desired to test the effect of sunlight or metals on the induction period, the milk was handled as previously described up to the point of churning. If metals were to be tested, 1″ x 2″ strips of the metals were placed into 750 cc. of cream and the cream pasteurized. Heating to 145° F., holding for 30 minutes and cooling at 40° F. required approximately one and one half hours. Control samples were pasteurized in the same water bath with the samples containing the metal strips. Cream samples which were exposed to sunlight were unheated. Raw samples of the same cream were protected from light and used as controls.

Conducting the tests: The tests to determine the induction periods were made in the following manner: Twenty-five cc. of the pure dry milk fat were melted and heated to approximately 65° C. One and one half cc. of a 0.025 per cent solution of methylene blue in absolute alcohol were then added to the

fat. A pipette was used to transfer approximately twenty cc. of the fat and dye mixture to the reaction cell (Fig. 2). The light in the right compartment was then turned on and readings on the milliameter taken at intervals until the reading had increased 1.5 milliamperes over the original reading. This reading was taken as the end-point of the fading of the methylene blue and the time required to reach the end-point is reported as the induction period Graphs 1 to 4 give some of the data in the form of curves. The position of the light and the photo-cell were kept constant. The results indicate that the maximum variation of duplicates in fading time of the methylene blue is approximately 3 per cent.

RESULTS

Graph 1 gives the results of exposing raw sweet cream which had been exposed to direct sunlight for two hours together with the control sample of the same cream which had been protected from light. The curve of Sample 202B shows the rate of reduction of the methylene blue in the control sample which has been arbitrarily designated as 100 per cent. The curve of Sample 207B gives the induction period of the treated sample which was 85.4 per cent of that of the control sample, or, in other words, direct sunlight, under the conditions of the experiments, markedly increased the sus-



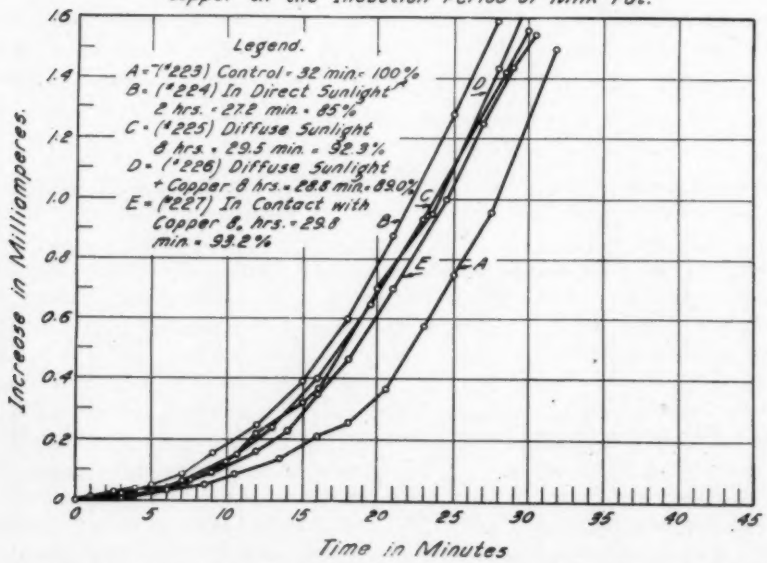
ceptibility of milk fat to oxidation. The flavor of the cream which had been exposed to direct sunlight was distinctly that of oxidized fat.

Graph 2 gives the induction period of raw fresh cream under the following conditions:

Curve A is the control sample which has been arbitrarily designated as	100%
Curve B direct sunlight 2 hours	85%
Curve C diffuse sunlight 8 hours	92.3%
Curve D diffuse sunlight 8 hours with copper	89.0%
Curve E treated as in Curve D except protected from light	93.2%

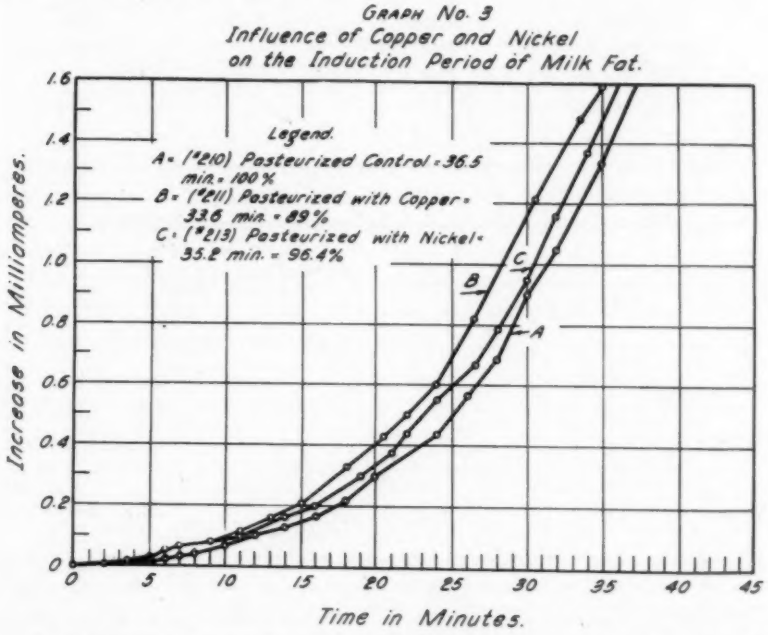
This graph shows that exposure of cream to direct sunlight decreased the induction period to a greater extent than did its exposure to diffuse sunlight, to diffuse sunlight and copper, and to copper alone.

GRAPH No. 2
Influence of Direct Sunlight, Diffuse Sunlight and Copper on the Induction Period of Milk Fat.



Graph 3 gives the results of pasteurizing two samples of cream, one in contact with copper and the other with nickel. Curve A shows the induction period of the control sample which is arbitrarily considered at 100 per cent. Curve B gives the induction period of the sample pasteurized with copper which was 89 per cent of the control. Curve C gives the induction period of the sample pasteurized with nickel which was 96.4 per cent of the

control. From Curve B one would conclude that copper had a marked influence in reducing the induction period of fat at while nickel, as shown in Curve C, had only a slight influence.

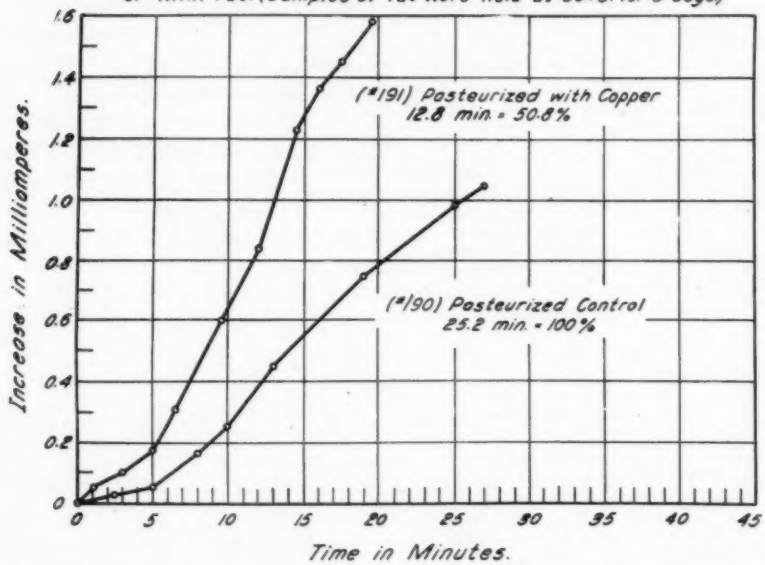


Graph 4 gives the results of an accelerated aging test of a control and a sample pasteurized with copper. To accomplish this, the pure fat samples were held in a 60° C. oven for five days. The curve of Sample 191 shows the induction period of the sample pasteurized with copper. The induction period was 50.8 per cent of that of the control sample. These results show that cream pasteurized in contact with copper produces pure fat that is markedly more susceptible to oxidation than fat that is not exposed to copper. In another accelerated aging test, using a different fat, the induction period was reduced to 75.3 per cent of the control.

Cream samples pasteurized in contact with a chrome-nickel-iron alloy of the 18–8 series with surface finishes, designated No. 1, No. 2, and No. 4, were tested and found to have no influence in changing the induction period of milk fat.

The influence of unsaturation of milk fat on the induction period: Hunziker, Mills and Spitzer (11) have shown that feeding cows rations high in linseed oil meal or cottonseed oil meal will greatly increase the iodine number of fat. Frazier (2) has fed cows such rations and has found that the milk

GRAPH No. 4
Influence of Accelerated Aging on the Induction Period of Milk Fat. (Samples of fat were held at 60°C. for 5 days.)



did not become tallowy more rapidly or to a greater extent when exposed to sunlight than milk produced from cows on rations that did not contain these ingredients. Triebold and Bailey (12) reporting their work on shortenings conclude "on the basis of the induction period, the susceptibility of shortenings to oxidative rancidity appears to be related to the unsaturation of the fatty acid glycerides." Eckles and Palmer (13) were able to increase the iodine number of milk fat approximately 15 units by feeding the cows on subnormal rations. No information was found in the literature that reported the change in induction period accompanying such a change in unsaturation of the milk fat.

Two cows (No. 403 and No. 478) of the Station herd were maintained on normal rations with the required amount of nutrients for milk production. The cows were in the third month of lactation, and were yielding approximately 40 pounds of milk per day. After samples were collected and tested for the normal period, the cows were reduced to submaintenance rations of 12 pounds of alfalfa hay per day. After 48 and 60 hours of this régime, other samples were collected for analysis. The cows dropped approximately 40 per cent in milk yield and showed loss in weight. The iodine numbers of the milk fat produced during these two régimes are given in table 1.

TABLE 1

Iodine numbers of milk fat produced from cows on normal and submaintenance rations

COW NUMBER	SAMPLE NUMBER	IODINE NUMBER	RÉGIME
403	208	31.1	Normal
403	200	48.1	Subnormal
478	209	36.6	Normal
478	201	54.2	Subnormal

The iodine numbers of the fats from Cows No. 403 and No. 478 were increased 17 and 17.6 units respectively during the submainaenance régime. The cows were apparently drawing upon their body fat while the submaintenance rations were being fed.

The induction periods of the fat secured from the milk produced from Cow No. 478 during the periods of normal and subnormal feeding were determined. These data are shown below:

TABLE 2

SAMPLE NUMBER	RÉGIME	IODINE NUMBER (HANUS)	INDUCTION PERIOD	PERCENTAGE OF NORMAL
209	Normal	36.6	27 min.	100.
201	Submaintenance	54.2	20 min.	74.2

It is evident from these results that the increase in the iodine number (Hanus) is accompanied by a shorter induction period. The iodine number was increased from 36.6 to 54.2 and the induction period was reduced 25.8 per cent. It would appear that any condition or feed that will greatly increase the unsaturation of milk fat will increase the susceptibility of the fat to become oxidized. The condition reported is an extreme one and under ordinary herd management would not be encountered to the same extent.

CONCLUSIONS

(1) The susceptibility of milk fat to oxidation due to different conditions may be measured by the photochemical method described by Greenbank & Holm with such modifications as have been described in this paper.

(2) Exposure of cream to direct sunlight, to diffuse light and to the action of copper gave definite increases in the susceptibility of milk fat to oxidation and the extent of the change was indicated by the method used. Direct sunlight, apparently, had the greatest influence. Nickel showed a slight influence, and a chrome-nickel-iron alloy of the 18–8 series had no influence on the susceptibility of milk fat to become oxidized.

(3) Milk produced from animals drawing upon their body fat by the consumption of submaintenance rations showed increases in the percentages of unsaturated fats, and increased susceptibility of the fat to oxidation.

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THE VALUE OF HAND STRIPPING AFTER MACHINE MILKING[1]

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INTRODUCTION

Hand stripping has been one of the most serious objections raised by dairymen to the use of milking machines. Petersen and Swenson (3) and Wallis *et al.* (5) have pointed out that little information is available as to whether or not stripping is necessary to maintain production. With the use of the combine milker, hand stripping is very inconvenient since the milk is handled entirely by machine. Some users have dispensed with hand stripping with apparently little ill effects. Others have massaged the udder while the machine was still operating in order to remove the last of the milk. The work reported here was carried out during the winter of 1931–32 to investigate this problem from three angles:

1. To find the amount of time spent in stripping and the amount of milk and fat secured in the strippings following machine milking.
2. To determine the effect of omission of hand stripping upon the production of machine milked cows.
3. To determine to what extent the amount of strippings can be decreased by procedures designed to increase the thoroughness of machine milking.

EXPERIMENTAL

I. *Time Required to Strip after Machine Milking and the Amount of Milk and Fat in the Strippings*[2]

The time required to strip was determined for each of 25 cows. The amount of milk and fat was found in the strippings of 23 of them. The group included 12 Holsteins, 5 Jerseys, 3 Guernseys, 4 Brown Swiss and 1 Ayrshire. The cows varied in age and stage of lactation, and their production ranged from 11 to 63 pounds of milk per day. The cows were milked twice daily. The milking machine was left six minutes on each cow. Previous work at this Station (2) has shown this to be about right for heavy milking cows. The time required to strip each cow was recorded for five or more milkings and averaged. Strippings were weighed and tested during a period of five days.

An average of 47 seconds per milking was required to strip after the machine. This time varied from 25 to 81 seconds with individual cows.

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[1] Journal paper No. J122 of the Iowa Agricultural Experiment Station, Ames, Iowa.

[2] The machine used in this trial was a De Laval Magnetic Milker.

The average amount of strippings obtained was 0.6 of a pound per milking or 1.2 pounds per day. Some cows gave as little as 0.1 of a pound per milking while the heaviest stripper averaged 1.7 pounds per milking. Between cows the amount of strippings was not closely related to the amount of milk produced, but with the same individual the amount of strippings tended to increase with advancing lactation. The fat test of the strippings of individual cows ranged from 2.8 to 13.5 per cent, with an average of 7.5 per cent for the 23 cows. Four and two-tenths per cent of the total milk produced and 7.3 per cent of the total fat produced were obtained in the strippings.

TABLE 1

Milk and fat production of cows not stripped after machine milking as compared to their production when stripped after machine milking

PRODUCTION		PERIOD I	PERIOD II	PERIOD III
		Stripped	Not stripped	Stripped
Group A (5 cows)	Milk lbs.	7773.0	6649.0	6068.0
	Fat lbs.	279.5	237.2	220.4
	Per cent fat	3.60	3.57	3.63
		Not stripped	Stripped	Not stripped
Group B (3 cows)	Milk lbs.	3800.0	3564.0	3359.0
	Fat lbs.	146.7	134.3	128.4
	Per cent fat	3.86	3.77	3.82

Summary of data

PRODUCTION OF 8 COWS IN 40 DAYS	MILK, LBS.	FAT, LBS.	PER CENT FAT
Stripped			
Av. of Group A in Per. I & III plus Group B in Per. II	10,485	384.2	3.66
Not stripped			
Av. of Group B in Per. I & III plus Group A in Per. II	10,229	374.8	3.66
Increase in production during period of stripping	256	9.5	
Av. increase per cow per day during period of stripping	.80	.03	
Per cent increase during period of stripping	2.5	2.5	

II. The Effect of Not Stripping after Machine Milking

Ten cows were divided into two groups as uniform as possible in regard to amount of strippings, total milk and fat production, breed, age, and stage of lactation. During the trial two cows were dropped from one of the groups for reasons not connected with the experiment, leaving only three animals in that group. The trial consisted of three 40-day experimental periods separated by transition periods of 5 days each. During each 40-day period the production of one group of cows which was not stripped after the milking machine was compared with that of the other group which was

hand stripped after the machine. The groups were alternated by the double reversal method. Each animal was stripped as soon as the milking machine was removed. One man did all the stripping to ensure uniformity. The machine-drawn milk and strippings of each cow were weighed, sampled and tested for butterfat separately at each milking during the first ten days of each 40-day period. During the latter 30 days of each period strippings were added to the machine-drawn milk and the tests were based on 5-day composite samples. All cows were stripped during the transition periods and weights and tests were taken at each milking. The milk and strippings were weighed to the nearest tenth of a pound on spring balance scales. A sampling tube was used for taking the composite samples. Fat tests were determined by the Babcock method.

The production of each group of cows during periods of stripping and not stripping are shown in table 1. The data were summarized by comparing the average of the first and third 40-day periods of the double reversal trial with the second. During the 40-day periods in which stripping was practiced the total daily production per cow amounted to 0.8 of a pound more milk and 0.03 of a pound more fat than during the corresponding period in which stripping was omitted. This was equal to an increase of 2.5 per cent in milk and fat production. The average butterfat test of the milk showed no change due to failure to strip. Figure 1 presents graphically the production records of the cows during periods of stripping and not stripping, and also during the 5-day transition periods previous to and following the 40-day period.

III. Practices Designed to Increase the Thoroughness of Machine Milking

Two methods of manipulation were tried in an effort to reduce the amount of strippings retained in the udder. The first consisted of thoroughly massaging the udder during the last two minutes of operation of the machine (the machine remained on each cow six minutes). In this method the teat cups or claw of the machine were not disturbed. The second method, designated in this article as manipulation of the teat cups, consisted of grasping the claw of the machine with the hand and exerting as much downward pressure during the last minute of milking as was possible without pulling the cups off the teats. This downward pressure was released at intervals, but no attempt was made to massage the udder further than resulted from the application and release of pressure on the teat cups.

The effect of massaging the udder was determined in a trial with three periods of 10 days each. Two groups of five cows each were used, the udders of one group being massaged while those of the other group were not. The groups were alternated by the double reversal method. Results were calculated by comparison of the average production of each group in

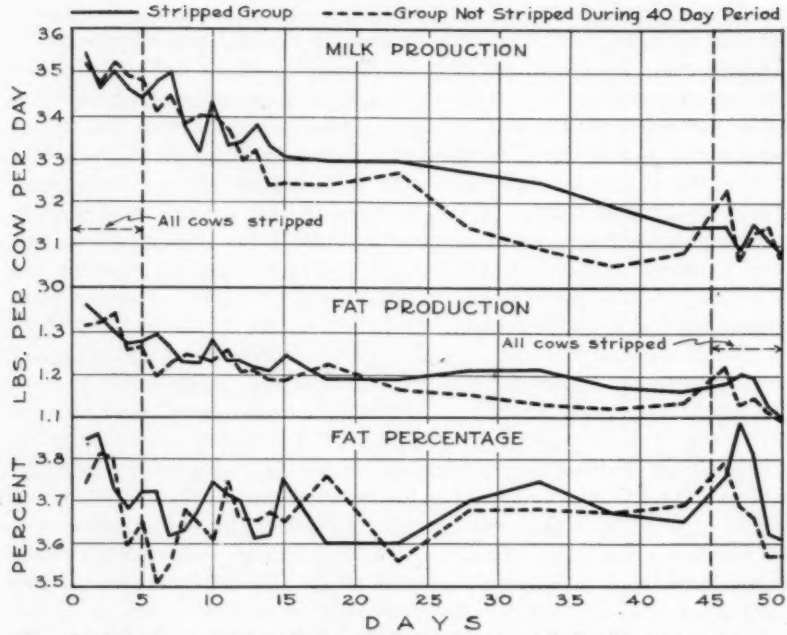

FIG. 1. THE AVERAGE DAILY PRODUCTION OF GROUPS OF 8 COWS STRIPPED AND NOT STRIPPED AFTER MACHINE MILKING.

Note: These figures represent the weighted average production during periods of stripping and not stripping. The production of the cows during the five days preceding and the five days following the 40-day period are also included.

TABLE 2

The thoroughness of machine milking as influenced by massaging the udder while the machine was operating

	GROUP (5 COWS IN EACH GROUP)	PRODUCTION OF GROUPS DURING EACH 10-DAY PERIOD								
		PERIOD I			PERIOD II			PERIOD III		
		Milk lbs.	Fat lbs.	% fat	Milk lbs.	Fat lbs.	% fat	Milk lbs.	Fat lbs.	% fat
Machine-drawn milk	A	1117	44.6	3.99	1028	41.8	4.07	1025	41.6	4.06
	B	1073	42.1	3.93	1051	41.6	3.94	1007	40.9	4.06
Strippings after machine milking	A	41	3.3	8.15	46	3.5	7.54	35	3.0	8.37
	B	55	3.3	6.03	26	2.2	8.34	41	2.9	7.02
Total production	A	1158	47.9	4.14	1074	45.3	4.22	1060	44.6	4.21
	B	1128	45.5	4.03	1077	43.8	4.07	1048	43.8	4.18

Group A massaged in periods I and III but not in period II.
Group B not massaged in periods I and III but massaged in period II.

Summary of data

		AV. PRODUCTION PER COW PER DAY LBS.		PER CENT OF TOTAL PRODUCTION	
		Massaged*	Not** massaged	Massaged	Not massaged
Machine-drawn milk	Milk	21.2	20.7	97.1	95.7
	Fat	.847	.833	94.1	92.7
	% fat	3.99	4.03		
Strippings after machine milking	Milk	.64	.94	2.9	4.3
	Fat	.054	.066	5.9	7.3
	% fat	8.29	6.98		
Total production	Milk	21.9	21.6		
	Fat	.901	.899		
	% fat	4.12	4.16		

* The group which was massaged includes the average of group A in periods I and III plus group B in period II.

** The group which was not massaged includes group A in period II plus the average of group B in periods I and III.

the first and third periods with that of the same group in the second period. Table 2 shows the average daily production of milk and strippings for the 10 cows during periods in which massaging was practiced and also during comparable periods when massaging was omitted.

A different method was used to determine the influence of manipulation of the teat cups on the thoroughness of machine milking. Ten cows were used in the trial which covered 15 days. The pail of the milking machine was suspended from a spring balance scale. During a 5-day preliminary period the amount of milk secured by the machine for each successive minute of milking was recorded. At the end of six minutes the milking machine was removed and the cow stripped. During the second 5-day period the machine was operated normally for the first five minutes but during the sixth minute the teat cups were manipulated. The weights of the milk secured by the machine during each successive minute and the weight of the strippings were recorded. In the third 5-day period the machine was operated normally for four minutes, then the teat cups were manipulated during the fifth minute. The machine was removed at the end of five minutes instead of at the end of six minutes. Weights of milk and strippings were recorded as in the preceding periods. Table 3 shows the average pounds of milk secured during each minute of milking and the amount of strippings obtained for each of the three periods.

DISCUSSION OF RESULTS

In this trial it was found that stripping after machine milking resulted in increased production. The average increase during the periods in which

TABLE 3

*The effect of manipulation (pulling down) of the teat cups on the completeness of machine milking**

		AMOUNT OF MILK OBTAINED BY MILKING MACHINE DURING SUCCESSIVE MINUTES OF OPERATION						STRIPPINGS AFTER MACHINE MILKING	TOTAL PRODUCTION
		Minutes							
		1	2	3	4	5	6		
Normal machine milking	Milk lbs.	3.2	4.7	3.1	1.3	.4	.2	.5	13.3
	% of total production	23.9	35.0	23.0	9.7	3.3	1.3	3.8	
Teat cups manipulated during 6th minute	Milk lbs.	3.1	4.5	2.7	1.3	.5	.7	.2	13.1
	% of total production	23.7	34.5	20.5	10.0	3.7	5.7	1.7	
Machine left on only 5 minutes. Teat cups manipulated during the 5th minute.	Milk lbs.	3.2	4.5	2.6	1.2	1.1	.....	.3	12.9
	% of total production	25.2	34.6	20.1	9.6	8.4	.....	2.2	

* Average pounds of milk per cow per milking. Each figure is the average production of 10 cows over 10 milkings.

stripping was practiced was 0.8 of a pound of milk and 0.03 of a pound of fat per cow per day. The average time spent in stripping a cow was 1.57 minutes per day. This means that for each hour spent in stripping the milker received 1.16 pounds of butter fat.

It must be pointed out that only relatively short periods (40-day) of non-stripping were used in this trial. What the effect would have been over a whole lactation period cannnot be definitely stated. It may be pointed out, however, that the production was decreased more during the latter part of the 40-day period than at the beginning of the period (Figure 1). Also, during the five days after the resumption of stripping the production of the previously non-stripped group practically paralleled that of the other group. These facts indicate that the loss by not stripping over a whole lactation might be greater than for the 40-day period. On the other hand, Woodward (7) found that incomplete milking did not lead to a rapid drying-off of the cows. He also reports that it did not alter the test of the milk which agrees with the results obtained in this trial.

Woll and Humphrey (6), although declaring that stripping is advisable, report that cows when not stripped for 12 weeks deereased in production no more rapidly than did cows which were stripped. Further work upon the effect of non-stripping is desirable before making too definite a statement regarding the ultimate effect of non-stripping upon production.

At least part of the milk and fat left in the udder when stripping is not practiced is recovered at the succeeding milking. This is shown by the

fact that milk and fat production were increased only 2.5 per cent by stripping, whereas the strippings left in the udders of the eight cows used in this trial constituted 4.4 per cent of the total milk production and 8.5 per cent of the total fat production. The recovery amounted to 46 per cent of the milk and 73 per cent of the fat left in the udder. Probably all the residual milk is actually recovered at the next milking but the presence of the milk causes the pressure to build up more rapidly in the udder. This increased pressure would depress secretion during the interim between milkings since the rate of secretion has been found by Ragsdale *et al.* (4) to be governed by the pressure in the udder. This conclusion is supported by the fact that the test of the milk is not altered by failure to strip and that a larger proportion of fat than of milk in the srippings was recovered.

M'Candlish (1) voices a popular opinion when he suggests that if a cow is stripped after machine milking she may retain a portion of her milk for the stripper instead of milking out completely with the machine. Results secured in this investigation do not bear out this statement. In fact, after a 40-day period without stripping the cows gave an average of 0.3 of a pound more strippings per day than after periods in which stripping was practiced. Merely omitting the stripping process for 40 days did not cause the cows to milk out more completely with the machine.

Certain difficulties encountered in this trial indicate that stripping after machine milking should be recommended as a safety factor. Three times during the trial the machine, due to improper attachment, failed to milk one quarter of the udder. This failure occurred even though a careful milker was operating the machine. By stripping after machine milking this failure was detected, but if stripping had been omitted it might have been overlooked. One cow was also encountered which did not milk readily by machine due to the partial obstruction of one teat. This cow gave as much as five pounds of strippings at a milking. Such a cow must, of course, be stripped after the milking machine. No serious cases of mastitis were encountered among the unstripped cows.

Though the trials relating to substitutes for hand stripping were not very extensive, they indicate that certain procedures will partially replace hand stripping. Manipulation of the teat cups for one minute reduced the amount of strippings to less than half the normal amount. Where stripping is very inconvenient manipulation would render machine milking more thorough and at the same time lessen the chance of mechanical failures of the machine escaping detection. However, neither process investigated was successful in securing all the milk that would have been obtained by stripping. Probably a combination of pulling downward on the teat cups and massaging the udder at the same time would prove more effective than either method used singly, but this was not attempted in the trial being reported.

SUMMARY

Stripping after machine miking required an average of 1.57 minutes per cow per day. One and two-tenths pounds of milk and 0.09 of a pound of fat, representing 4.2 per cent and 7.3 per cent respectively of the day's total production were secured in the strippings.

During periods in which stripping after machine milking was practiced the production of milk and fat was 2.5 per cent greater than in periods in which the stripping was omitted. Calculations show that not stripping resulted in the loss of 54 per cent of the milk and 27 per cent of the fat that would have been obtained in the strippings. No change in the fat percentage of the milk was caused by not stripping. For each hour of labor spent in stripping 1.16 pounds of fat were secured.

Massaging the udder during two minutes of the time the machine was operating decreased the amount of strippings 33 per cent. Manipulation (pulling down) of the teat cups for one minute caused a 55 per cent reduction in the amount of strippings. The cows were milked more thoroughly by the machine in five minutes if manipulation of the teat cups was practiced for one minute (the last) than in six minutes of normal machine miking.

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American Dairy Science Association Announcements

ANNUAL MEETING

Ithaca and Geneva, New York, June 26 to 28, 1934

It is expected that many members will again combine their summer vacation and attendance at the annual meeting. Entertainment is being planned for the women and children. There are many points of scenic and historic interest in this vicinity. There will be a registration and information desk in the Dairy Industry Building in Ithaca on June 25 and 26.

Members are urged to promptly send in titles of papers to J. M. Sherman, Ithaca, N. Y., as it is hoped that the program will be completed in time to appear in the June number of the JOURNAL OF DAIRY SCIENCE.

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